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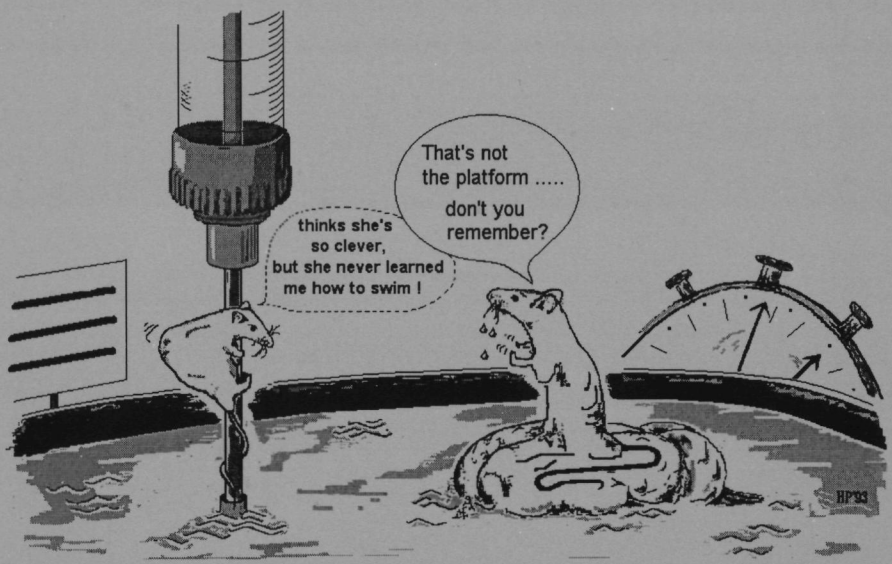
STRIATAL DOPAMINE IN LEARNING AND MEMORY

Investigations on the role of dopaminergic activity
in the ventral and dorsal striatum
in diverse learning and memory tasks
in rats



G.E. Ploeger

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Een wetenschappelijke proeve op het gebied van
de Medische Wetenschappen

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aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen
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Het werkelijke in ons zwijgt,
het aangeleerde is spraakzaam.

Kahlil Gibran

I sometimes feel, in reviewing the evidence
on the localization of the memory trace,
that the necessary conclusion is
that learning just is not possible
It is difficult to conceive of a mechanism
which can satisfy the conditions set for it
Nevertheless, in spite of such evidence against it,
learning does sometimes occur

Karl S Lashley,
from In search of the engram
Society of Experimental Biology Symposium, no 4
Physiological Mechanisms in Animal Behaviour,
Cambridge University Press, pp 454-482, 1950

CONTENTS

LIST OF ABBREVIATIONS

6OHDA	6-hydroxydopamine
AChE	acetylcholine-esterase
AD	distilled water
AGI	anogenital investigation
AMP	amphetamine
APO-SUS	apomorphine susceptible
APO-UNSUS	apomorphine unsusceptible
CER	conditioned emotional response
CR	conditioned response
CS	conditioned stimulus
DA	dopamine
DPI	(3,4-dihydroxyphenylimino)-2-imidazoline
DS	dorsal striatum
FRP-AAC	fixed response pattern of adjacent arm choices
GABA	gamma-amino-butyric-acid
HAL	haloperidol
icv	intracerebroventricular
ip	intraperitoneal
ITI	intertrial interval
LTM	long-term memory
LTP	long-term potentiation
MWM	Morris water maze
NA, NE	noradrenaline
NAC	nucleus accumbens
PCx	piriform cortex
RAM	radial arm maze
RM	reference memory
sc	subcutaneous
SIT	social investigation time
SNpc	substantia nigra pars compacta
SNpr	substantia nigra pars reticulata
SR	social recognition
ST	successful trial
STM	short-term memory
STWE	successful trial without error
TM	T-maze
UR	unconditioned response
US	unconditioned stimulus
VS	ventral striatum
VTA	ventral tegmental area
WM	working memory

CHAPTER 1.
GENERAL INTRODUCTION

CONTENTS OF CHAPTER 1

1.1 LEARNING AND MEMORY

It is difficult to give a precise definition of learning or memory. In general, learning relates to an organism changing its behaviour, in a relatively permanent way, in order to adapt to some change in the environment (see McFarland, 1987). However, this process is always inferred afterwards, i.e. we may infer that an animal has learned something out of an experience when we observe that a particular alteration in its behaviour has taken place. Not all changes imply learning. For example, activities due to an altering state of motivation (hunger, thirst) or (sexual) maturation are not consequences of learning processes and neither are behavioural changes because of fatigue. Memory refers to the persistence of a learned response over time and memory processes involve neuronal mechanisms that sustain acquired information or responses.

Psychology has a long tradition of studying learning and memory processes at a behavioural level, resulting, among others, in a large number of descriptive distinctions. Since appropriate neurobiological techniques became increasingly available, attempts have been made to understand learning and memory also at physiological, cellular or even molecular levels.

Phases

In the process of learning and memory we discriminate the actions (Squire, 1987; Heise, 1981) of acquisition of new information (i.e. learning; in a one-trial learning paradigm or during a course of several training trials); consolidation of the acquired information (a dynamic process which is assumed to take place over some period of time immediately after the training-trial); the retention or preservation of stored information (for some, more or less measurable, period of time: the so-called retention-interval); the retrieval of the learned information (as shown by the actual expression of the learned response on one or more test-trials); the phases of forgetting the information or extinction of a learned response (figure 1).

The design of the procedure of experimental intervention should carefully consider which of these phases may be altered by the manipulation, depending on when the intervention is carried out.

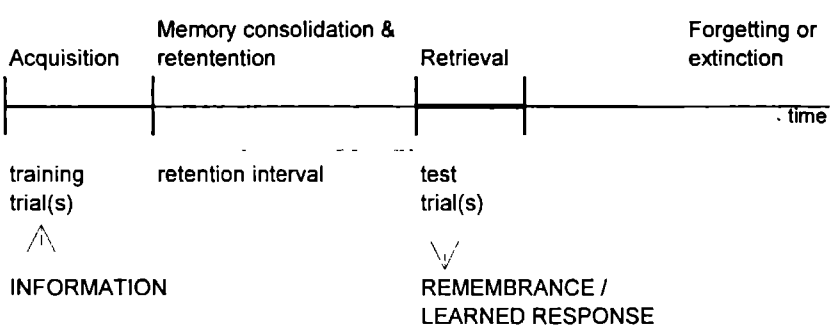

Figure 1. Phases in the process of learning and memory

Simple and complex learning

Historically, a distinction has been made between simple versus complex learning (McFarland, 1987, Squire, 1987) In fact, different kinds of learning and memory processes can be more or less arranged in order of increasing complexity

Habituation and sensitization constitute examples of simple learning Repeated presentation of one stimulus may result in a decreased responsiveness (habituation) The habituated response will reappear if the stimulus is withheld for a long period of time Furthermore, habituation to one stimulus may generalize to another, similar stimulus On the other hand, repeated presentation of an intense or significant stimulus may result in an increased responsiveness to a wide variety of neutral stimuli

Conditioning Classical and instrumental (operant) conditioning, forms of simple associative learning, already bear more complexity A huge amount of research has been carried out regarding conditioning, which has profoundly influenced the research on learning and memory Therefore and because dopamine appears to play a role in instrumental behaviour (see § 1 2), a brief discussion on these two topics will follow here

A (biologically) significant unconditioned stimulus (US) elicits a specific unconditioned response (UR) In classical conditioning, pairing or association of a previously neutral conditioned stimulus (CS) with the US will lead to the CS alone to be able to elicit the response, which then becomes the conditioned response (CR) A well-known example of classical conditioning is Pavlov's experiment on conditioned salivation in dogs

In the most simple form of instrumental conditioning, the occurrence of a motivationally significant event is contingent on the performance of a specific response of the organism This significant event is said to reinforce (strengthen) the response, resulting in an increased probability that the animal will perform that response The magnitude of the increase in probability depends on how reliably performance of the response and the significant event coincide (contingency) Among many others, Thorndike (1933) and Skinner (1938) have investigated instrumental conditioning its most simple version involves an animal learning to open a door or press a lever, whereafter food becomes available (in Thorndike's puzzle box and the Skinner box, respectively) In the case of classical conditioning, the presentation of the US is sometimes said to reinforce the occurrence of the CR in response to the CS One important difference between classical and instrumental conditioning appears to be that the latter is more readily modified by the consequences of the performed response

The notion of reinforcement is still debated in literature (see White, 1989, White and Milner, 1992, Lieberman, 1990)

In the traditional view (of the associationists and the behaviourists), reinforcement refers to the capacity of certain important events to enhance the storage of information or to strengthen the connection between a stimulus and a response For example, general emotional stimulation (by approval or disapproval) may facilitate recall of a list of words in human subjects (see White, 1989) Furthermore, post-training delivery of food can improve retention in a passive

avoidance test (Huston and Oitzl, 1989) This function of reinforcers implicates that no meaningful relationship between the reinforcing event and the stored information needs to exist Thorndike (1911, 1933) defined this memory enhancing property of reinforcers in his "Law of Effect" (see White and Milner, 1992) Thus, in the case of the instrumental conditioning in his puzzle box, Thorndike emphasized that receiving food "stamped in" an association between the stimuli of the box and the response of escaping from it

However, cognitive theorists (e g Tolman et al , 1932, Bindra, 1978, Bolles, 1972) recognized the motivational role of reinforcement A reinforcer may induce a certain expectational or motivational state in the animal, that is capable of eliciting a wide range of preparatory and consummatory behaviours in order to obtain this reinforcer The unconditioned reinforcing stimulus is defined as the primary reinforcer, also called the primary incentive stimulus or reward Association of a primary reinforcer with a previously neutral stimulus results in this latter stimulus to acquire the capacity of motivationally eliciting the same group of behaviours Such a stimulus is then called a conditioned motivational reinforcer, also termed the secondary reinforcer or the conditioned incentive motivational stimulus The process of association is termed incentive motivational learning (Beninger, 1983, Salamone, 1992)

The term reinforcement is often used interchangeably with the term reward However, the notion of reward goes a longer way back in history The Epicurean philosophers already described that behaviour of individuals is determined by the tendency to maximize pleasure and minimize pain (see White, 1989) In modern psychology an operational model of reward and its counterpart aversion is used the operational definition of reward is approach, whereas aversion is operationally defined as withdrawal (Young, 1962) A positively reinforcing stimulus is assumed to have rewarding properties The hedonic view of reinforcement is based on this assumption, stating that stimuli are reinforcing because they are rewarding, i e inducing a state of pleasure (hedonia) However, pleasure and pain are subjective sensations and such states of mind are difficult to identify in organisms So, this view is rejected by many authors and replaced by other theories on drive reduction or activation of consummatory responses, among others (see Salamone, 1992)

Traditionally, associative forms of learning were regarded as fundamentally simple processes, just based on the formation of associations between contiguous events Decades of research on a large variety of conditioning tasks, however, have shown these processes to be governed by a complexity of factors and rules, leaving still unresolved issues Associationism also assumed that complex ideas were built up from associating simple ideas and according to the related movement of behaviourism only the visible and overt behaviour was allowed to be the object of investigation, because of the impossibility of observing mental states In contrast to these traditions, cognitive behaviourists have pointed to the existence of internal cognitive processes, such as attention, expectations, motivation, discrimination and insight, and to the importance of these processes even in associative learning For these scientists, abilities of organisms to impose structure (categories) on their experiences by means of internal processes are needed to explain the complexity and flexibility of the behaviour of both humans

and animals Lieberman (1990) exhaustively discussed these contrasting scientific views in memory research

Complex learning Perceptual or discriminative learning, conceptual learning, cognitive mapping and rule learning (learning sets) are examples of learning of high complexity, that is not easily explained in terms of simple, fixed associations between stimuli and responses Discriminative learning includes brightness discrimination and visual discrimination between forms The ability to discriminate may develop in the absence of reinforcement, as a result of 'mere exposure' to the stimuli and it is assumed to result from an active process of building up a set of descriptions of relevant, differentiating features of particular stimuli and situations Rule learning involves the acquisition of information on complex (sets of) rules governing the sequence of occurrence of events

Cognitive or spatial mapping has its own history of research Apart from a philosophical and psychological debate on the notion of space, research has been carried out on the ability of organisms to find their way back home or to regain buried food (see O'Keefe and Nadel, 1978) A well-known and extensively studied example of such an ability is the phenomenon of bird navigation (homing, migration, e g Keeton, 1974)

Furthermore, many experiments on maze behaviour in rats have been executed, by proponents of the opposing theories on response learning and place learning The behaviouristic view assumes that, in learning to solve a maze problem, the animal acquires the ability to execute a chain of responses that are directed by proprioceptive stimuli (stimulus-response association or habit formation, leading to a sequence of turn left - turn right - etc) However, several nice maze experiments by Tolman (Tolman and Honzik, 1930, Tolman, 1948) and Hebb (1949) showed place-learning tendencies Animals demonstrated a sense for direction, and after having learned to locate a specific object in a particular place, rats preferred to approach a different object in the previously correct place rather than to approach the same object in a different place So, Tolman and Hebb supposed that animals exposed to a novel environment would construct map-like representations (cognitive maps) of that environment in their brain

In general, rats appear to learn running to one place from different directions more readily than making the same turn to different places This finding supports the place learning hypothesis Later maze experiments by Olton (Olton and Samuelson, 1976) have provided additional evidence rats quickly learn to collect food pellets from maze arms in an efficient way and without making obvious fixed responses

Among place learning theorists, the importance of distal cues (outside the apparatus or extra-maze), relative to local cues (within the apparatus or intra-maze), has been emphasized Distal cues provide hints on directions and remain relatively constant when the animal moves about its local environment Distal cues cannot precisely point to specific places

O'Keefe and Nadel (1978) further elaborated the theory on spatial mapping abilities Different strategies, either using simple routes or more complicated maps, may indeed be employed to find a specific location

The use of routes requires instructions for the execution of (a fixed sequence of) movements, it is a simple and fast but inflexible strategy This so-called taxon strategy include two types of 'instructions' guidance by a clearly available and detectable stimulus or landmark that is to be avoided or approached (also called cue strategy) and orientation of the body axis (also called response strategy) Both strategies are supposed to rely on the activation of egocentric spatial systems

The construction and use of cognitive maps about the spatial relations between objects in the external world provides a more complex but stable and flexible way of localization It enables an organism to find a goal in a specific place from different places O'Keefe and Nadel termed this the locale strategy, it is assumed to rely on the activation of allocentric spatial navigation systems (O'Keefe and Nadel, 1978)

Stages and types of memory

The brain processes events or information of very different kinds leading to the formation of memories containing the information Nowadays, theory on information processing is based on analogy to computer technology and comprises the stages of coding the incoming information, its (local) storage and the retrieval of memories from storage With regard to the organization of memory itself we may distinguish between several stages and types of memories (Squire, 1986) Classifications have arisen from experimental or cognitive psychology, human neuropsychology and animal neurobiological studies

One important division splits memory into the stages of short-term (STM) or recent and long-term (LTM) or remote memory Short-term memory refers to a capacity-limited system that retains information only temporarily (for seconds or minutes) before it becomes incorporated or transferred into a more stable and potentially long-term storage system James (1890) already distinguished primary from secondary memory, more or less comparable to the division in STM and LTM A remarkable example of the separation between STM and LTM stems from human neuropsychology The well-known patient H M , who became amnesic after neurosurgery to relieve severe epilepsy, still showed (aspects of) the ability to retain information in STM, while he appeared to be unable to acquire new information and hold it over a longer period of time (LTM), despite normal vocabulary, language skills and IQ (Milner et al , 1968)

Another distinction can be made between working (WM) versus reference (RM) memory Working memory denotes a memory buffer in which to maintain information that is of only temporary value, e g during the time of one trial Information valid for a longer period of time may then be held in reference memory The concepts of WM and RM can be studied in a radial arm maze in which a number of arms, always the same over the whole period of training, are never baited, whereas an animal needs to remember within one trial which arms it

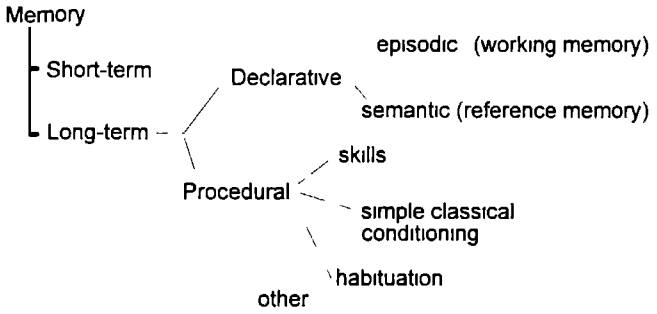

Figure 2. A tentative taxonomy of memory (adapted from Squire, 1986)

has already visited Recently, the working memory system is thought of as a collection of multiple temporary capacities, based on different modalities (see Squire, 1987)

A further classification of long-term memory (deriving from human studies) may be into the two types of declarative and procedural memory Amnesic patients often show unimpaired learning and memories in distinct tasks In general, these people are perfectly capable of performing even complex tasks, while at the same time they seem to be unaware of having been in the test before Declarative memory thus concerns explicit information (including facts, events, times, places), that is directly accessible to conscious recollection and that can be declared in a proposition or an image Acquisition of perceptual-motor skills leads to implicit procedural knowledge, only accessible through engaging these skills (Squire, 1987, 1986)

Next, declarative memory may be subdivided into episodic and semantic memory (Tulving, 1983) Episodic memory records information about dated events in an individual's life (building up someone's autobiography) Semantic memory, on the other hand, refers to knowledge of the world (facts, concepts, vocabulary), without relation to temporally landmarks

Together, the above standing distinctions may be used to compose a tentative taxonomy of memory (see figure 2, from Squire, 1986) Apart from the distinctions mentioned sofar, many others have been put forward by researchers believing memory to fall apart in at least two, often opposing, systems Examples include the distinction between memory versus habit (Mishkin et al , 1984), representational versus dispositional memory (Thomas, 1984), locale versus taxon memory (O'Keefe and Nadel, 1978) and explicit versus implicit memory (Graf and Schacter, 1985) Often, effects on distinct kinds of learning and memory related to damage in specific brain areas in humans are at the origin of imposing these categories onto memory

Localization

Naturally, a major issue in the study on learning and memory processes is the debate on where memory is localized and which brain areas contribute to the processes of learning The complete set of changes in the nervous system representing the stored memory is commonly termed the engram Historically, two contrasting theories exist with regard to where the engram is located the so-called localizationist versus the distributed view (Squire, 1987)

According to localizationist scientists, distinct and identifiable localized parts of the brain have distinct behavioural functions Experimental research supported this view, demonstrating that

localized brain lesions or stimulation produce specific effects on language or vision or motor movements, etc (Luria, 1966) Consequently, particular brain areas would be involved in representing particular memories

The distributed view states that behaviour and mental activity arise from the integrated activity from the entire brain Part of the distributed theorists favoured the field theory, for instance scientists from the Gestalt psychology (e g Kohler, 1940) In field theory, behavioural and mental function correspond to how electrical activity is distributed over large areas of the cortex, and not to any specific neuronal connections activated by the activity

Others, more empirical scientists, although not supporting the field theory, also attacked the localizationist view For example, Lashley (1929, 1950) spent many years, training lesioned rats to run through a maze, on attempting to identify any particular brain region as special or necessary to the formation of the maze habit He failed to do so Instead, he concluded that the reduction in learning ability is "the same, quantitatively and qualitatively, after equal lesions to diverse areas" (Lashley, 1929) Thus, he postulated the theory of memory being equivalently distributed over brain areas (functional equipotentiality)

Reconciliation of these views points to the experimentally supported notion that no separate areas exist where an entire memory (engram) is stored, but that representation of aspects of a memory may indeed be highly specifically localized Memory is distributed in the sense that many areas of the brain are involved Consequently, questions to be asked include which brain regions are involved in learning and memory processes, how they contribute and to what degree they are essential to these processes

A huge amount of experiments have been carried out regarding the specific brain areas that are somehow involved in specific kinds of learning and memories Investigations have shown which neural pathways are involved in the acquisition of, for instance, the acoustic startle reflex (habituation) in mammals (see Davis et al , 1982) or which areas are necessary for maintaining the conditioned nictitating membrane/eyeblink response in the well-trained rabbit (see McCormick and Thompson, 1984) It is expected to find the essential neural modifications underlying long-term behavioural change in those regions that are minimally required for maintenance of the response

Human neuropathology may provide data on effects of brain damage in diverse areas Specific neural damage can induce particular memory impairments in humans, often resulting in a division in two opposing kinds of memories A number of these divisions stems from a classification based on the effects of damage to the temporal lobe, especially the hippocampal formation Results obtained from testing amnesic patients, like the above mentioned H M , showing preserved abilities to acquire motor skills, have suggested the distinction between declarative and procedural learning and memory, with the former kind linked to a well-functioning hippocampus

Animal experiments have further supported dichotomic divisions in memory, related to specific brain areas For instance, studies employing lesions in the hippocampus provided evidence for a role of the hippocampus in WM (Olton and Papas, 1979) Allocentric spatial navigation is

proposed to depend on a well-functioning hippocampus (O'Keefe and Nadel, 1978) Mishkin linked his division in memory versus habit to the cortico-limbic versus the cortico-striatal system, respectively (Mishkin et al , 1984) Today, numerous learning and memory tasks for animals and humans are used to examine the question which specific brain areas contribute to the processing of information, the acquisition of task-specific responses, or which areas are necessary for the maintenance of the information or the response

Storing or representing information ultimately occurs at the cellular and molecular level (synaptic plasticity) Mechanisms such as a depressed synaptic transmission in the habituation of the withdrawal reflex in the marine snail *Aplysia* (Kandel, 1976) and a long-lasting increase in the strength of a synaptic response to particular electrical stimulation (long-term potentiation (LTP)), among others found in the hippocampus and the nucleus accumbens and possibly related to learning (Bliss and Lomo, 1973, McNaughton and Morris, 1987, Colley and Routtenberg, 1993, Mulder et al , 1993), have been described

1.2 THE INVOLVEMENT OF DOPAMINE IN PROCESSES OF LEARNING AND MEMORY

Dopamine has been implicated in a diversity of learning and memory processes Numerous studies have reported on various aspects in diverse learning and memory tasks that are or are not sensitive to dopaminergic treatment

General memory enhancement

Post-training stimulation of the dopaminergic activity can have a memory enhancing effect

So, a post-training injection of amphetamine (AMP), a known stimulant of dopaminergic activity, has been shown to facilitate memory consolidation, as expressed by improved retention during the test trial (see Carr and White, 1984)

Retention of a tone-shock association, as measured by the level of suppression of drinking, was significantly improved after injection of AMP immediately following the tone-shock presentation (Carr and White, 1984) Post-training intraventricular injections of DA also improved retention in a passive avoidance task (Haycock et al , 1977) Reversely, inhibition of the dopaminergic activity immediately after training, e g by means of the antagonist chlorpromazine, disrupted retention of a passive avoidance task (see Carr and White, 1984)

The memory improving effect upon systemic application of AMP has been replicated applying micro-injections of AMP into the dorsal striatum (see § 1 3) (Carr and White, 1984)

Furthermore, AMP has been demonstrated to improve memory retrieval Pre-test application of AMP attenuated forgetting in mice, that was induced by several sources (pharmacological, time-interval) (Quartermain et al , 1988a, Quartermain et al , 1988b)

Stimulus-stimulus association

Dopamine appears nòt to be essential for the establishment of the association itself between two stimuli (classical conditioning)

During the first experimental phase, rats drugged with neuroleptics (DA antagonists) were presented with paired stimuli (e g light ↔ shock) in a one-trial avoidance training The animals failed to learn the avoidance response However, during the second undrugged phase, these animals showed clear signs (a conditioned emotional response, CER) upon presenting the light, that the association between the light and the shock had been made (see Beninger, 1983, Beninger et al , 1980)

Reward-related instrumental learning

Dopamine has been demonstrated to affect instrumental responding It may be involved in the acquisition as well as in the maintenance or performance of a learned response However, different studies sometimes yield contrasting effects The reader is referred to reviews on studies of instrumental behaviour by Beninger (1983), Salamone (1992) and Blackburn and coworkers (1992)

DA receptor blockers have been shown to impair the acquisition of avoiding an aversive stimulus (shock), without affecting the ability of the animal to escape it (see Beninger, 1983, Beninger, 1989, Blackburn et al , 1992) The acquisition of lever pressing for food reward is also disrupted by neuroleptics (Wise and Schwartz, 1981)

Furthermore, neuroleptics induce a gradual decline over time in the performance of the response in well-trained animals (Wise et al , 1978, see Beninger, 1983)

The effects of dopamine on instrumental behaviour have often been related to reward Several tasks are used for measuring reward electrical self-stimulation, drug self-administration and diverse place preference tests All three paradigms have been shown to be sensitive to manipulation of the dopaminergic activity (Carr and White, 1983, Hoffman and Beninger, 1988, Wise and Bozarth, 1981, White, 1989, see Beninger, 1989) For instance, self-stimulation can be reduced by dopaminergic antagonists (see White, 1989) and pairing AMP with one of two compartments results in a preference for the AMP-paired environment (Hoffman and Beninger, 1988)

In addition, dopamine is implicated in conditioned reward In the conditioned reward experiment, a neutral stimulus (the CS, e g a tone) is paired to a rewarding unconditioned stimulus (the primary reinforcer, e g food) during the first phase The animal is only required to consume the rewarding stimulus during the pairing trials In the subsequent test session, it is examined whether the previously neutral stimulus has become a conditioned motivational stimulus, i e whether it has acquired response eliciting capacities Two levers are presented, one of which produces the conditioned stimulus whereas the other has no consequences Normal rats appear to be prepared to press the lever producing the conditioned stimulus (the tone) (without ever receiving the primary reward upon lever pressing!) Thus, this stimulus has become a conditioned reward, also called a secondary, conditioned reinforcer Neuroleptic treatment during the pairing sessions results in a decrease in the conditioned reward effect (Beninger and Phillips, 1980)

Secondary reinforcement (or incentive motivational learning or conditioning) has been extensively studied by Robbins and coworkers, showing that dopaminergic activity, especially within the nucleus accumbens (see § 1 3), is involved in this phenomenum (Robbins et al , 1983, Taylor and Robbins, 1984, Everitt et al , 1989, see also Cador et al , 1991)

Complex learning

Dopamine has been shown to affect more complex learning processes, such as spatial mapping and maze learning Also in these cases, conflicting results have been published
Disruptive effects of dopaminergic treatments on spatial localization of a hidden platform in the Morris water maze (see § 1 5) have been reported Depletion of dopamine by means of extensive 6OHDA lesions (specifically destroying catecholaminergic nerve terminals) appeared to block spatial learning (Whishaw and Dunnett, 1985) However, non-spatial localization of the platform also was deteriorated in this study In contrast, a different study on the effects of 6OHDA lesions (Hagan et al , 1983) demonstrated sufficient learning about the location of the platform as measured in the retention test Application of dopaminergic agents has been shown to affect mainly the acquisition phase in the Morris maze task (Scheel-Kruger et al , 1990, Taghzouti et al , 1987)
Effects of dopaminergic treatment on radial arm maze (RAM) performance have been examined Impaired acquisition of the standard version of the RAM (see § 1 5) after haloperidol (HAL) injection (systemic as well as into the nucleus accumbens) has been reported (McGurk et al , 1989, Taghzouti et al , 1987) Furthermore, lesions of the striatal dopaminergic areas have been shown to affect aspects of radial maze learning or performance (Schacter et al , 1989, Cook and Kesner, 1989, Packard and White, 1990, Packard et al , 1989), while others are spared (Cook and Kesner, 1988) It should be noted that in some of these RAM studies (Packard et al , 1989) animals were trained on a reinforced stimulus-response association Thus, it is important to distinguish between the aspects that are affected and the aspects that are left intact after dopaminergic treatments

Dopamine in learning and memory

In this paragraph, we have sofar provided examples of effects of dopaminergic manipulation in diverse learning and memory tasks The question remains whether dopamine mediates (a) common aspect(s) in learning and memory
Some authors have reviewed studies on the involvement of dopamine in instrumental responses (Salamone, 1992, Blackburn et al , 1992) It may be concluded from these reviews that dopamine from the telencephalic area (see § 1 3) potentiates the ability of (cortically processed) significant stimuli to induce the execution of (complex) motor responses
We will extend this view on the role of dopamine with a differential involvement for dopamine in the dorsal versus the ventral striatum in behaviour (see § 1 4) Prior to this, an overview of the anatomy and neurochemistry of the telencephalic striatum will be given

1.3 A BRIEF SURVEY ON THE ANATOMY AND NEUROCHEMISTRY OF THE VENTRAL AND DORSAL STRIATUM

Dopamine is present in the striatal areas. The striatum of the rat can be divided into two parts, based on their specific inputs and outputs: a *dorsal* part, the neo- or dorsal striatum (which is called the nucleus caudatus/putamen complex in humans), and a *ventral* part, that encompasses the ventromedial part of the neostriatum, the nucleus accumbens and the olfactory tubercle (Heimer et al., 1982). Each of these regions is characterized by its own pattern of af- and efferents. Both the dorsal striatum and the nucleus accumbens of the ventral striatum are considered in this thesis; throughout the text, however, the latter area will often be named as the ventral striatum as opposed to the dorsal striatum.

The striatal areas lie in the telencephalic basal ganglia, positioned in the forebrain. On the one hand they are considered to be part of the extrapyramidal motor system (see, however, the commentary of Côté and Crutcher, 1985). On the other hand, these areas are connected to several cortical and subcortical structures involved in cognitive and limbic funtions.

Dopamine can furthermore be found in mesolimbocortical areas (like the prefrontal cortex, hippocampus, amygdala) and in the tuberoinfundibular pathway; these structures are disregarded in this thesis.

Nucleus accumbens

The nucleus accumbens is positioned rostroventrally to the dorsal striatum; cytoarchitectonically the transition to the dorsal striatum is rather indistinct.

The accumbens is an important dopaminergic area, receiving innervation primarily from dopamine (DA) cells in the mesencephalic ventral tegmental area of Tsai (VTA; also labeled

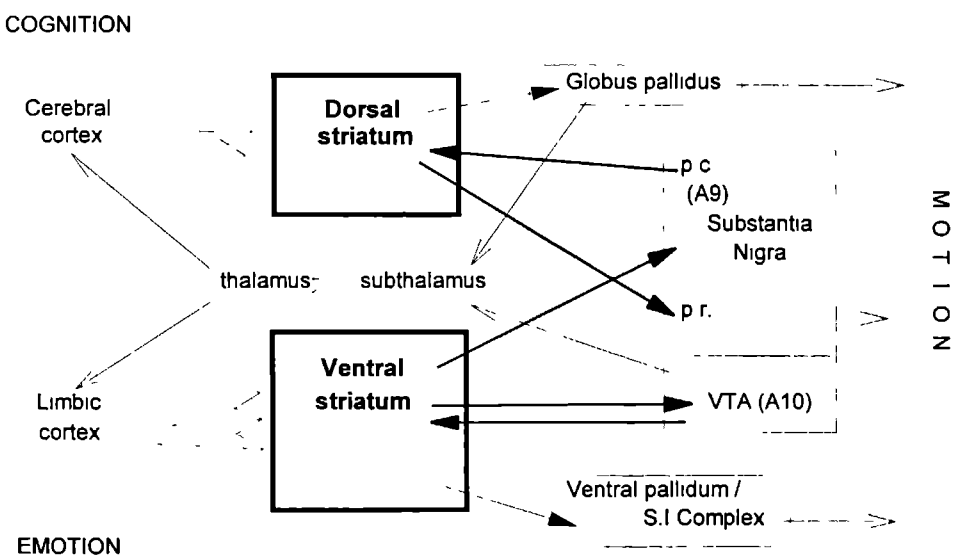

Figure 3. Schematic representation of a subset of the anatomical connections towards and from the striatal area.

as the A10 cell-group (Ungerstedt, 1971), figure 3) These dopaminergic fibers ascend through the medial forebrain bundle The VTA itself receives input a o from serotonergic and noradrenergic brainstem areas (non-specific arousing sensory stimulation) and from forebrain structures like the nucleus accumbens (feedback loop), prefrontal cortex, septum, amygdala and ventral pallidum (more specific processed information) (Scheel-Kruger and Willner, 1991, Kalivas, 1993, see Oades and Halliday, 1987 for a review on the VTA) A small contribution to the dopaminergic innervation of the accumbens is made by the A9 dopamine cell-group in the substantia nigra (pars compacta) (see Vrijmoed-De Vries, 1985, Fallon and Moore, 1978, Swanson, 1982) Several distinct dopamine receptors have been characterized (see below)

The nucleus accumbens also contains noradrenergic nerve terminals, although this neurotransmitter is present in lower quantities than the transmitter dopamine (Allin et al , 1988) Both α- and ß-noradrenergic receptors have been found, which appear to be modulated by noradrenaline from the ventral noradrenergic bundle and the dorsal noradrenergic bundle, respectively (Cools et al, 1991)

Furthermore, the nucleus accumbens receives afferents from various cortical and subcortical limbic structures (Groenewegen et al , 1991, Phillipson and Griffiths, 1985, Scheel-Kruger and Willner, 1991) The major allocortical input is from the hippocampal formation, in particular the subicular area and to a minor degree the CA1 region, descending through the fimbria and precommissural fornix (see figure 3, Groenewegen et al , 1987, Kelley and Domesick, 1982) Amygdalar input, traversing via the stria terminalis, arises primarily from the basolateral nucleus, whereas the central, medial and cortical nuclei of the amygdala provide a minor contribution to the projection to the accumbens Inputs further come from prefrontal, perirhinal and entorhinal cortices and from specific limbic related, midline thalamic nuclei The (sub)cortical projections appear to be glutamatergic (using the neurotransmitters glutamate/aspartate) and excitatory (Walaas, 1981, Yang & Mogenson, 1985, Yim and Mogenson, 1986)

All the afferents project to the nucleus accumbens in a distinct topographic order Concentrations of both dopamine and noradrenaline seem to increase towards the caudal part of the nucleus accumbens (Allin et al , 1988) The prefrontal cortex only projects to the anterior nucleus accumbens, which further receives afferents from the dorsal (or septal) subiculum and CA1 area (medial and lateral NAC) and from the basolateral amygdala (medial NAC) The ventral (or temporal) subiculum sends fibers to the more posteromedial NAC (Phillipson & Griffiths, 1985, Groenewegen, Vermeulen-VanderZee, Tekortschot, & Witter, 1987) Overall, Phillipson and Griffiths concluded that the largest volume of inputs is projected to the anteromedial accumbens, where output from the subiculum and the hippocampal CA1 region, the entorhinal and prefrontal cortices and the amygdala converge with output from the VTA and midline thalamus

Two main groups of cells are found within the nucleus accumbens medium-sized spiny GABAergic output neurons (Smith and Bolam, 1990) and large spine-poor or aspinous

cholinergic interneurons (Phelps and Vaughn, 1986) Medium-sized aspinous GABAergic interneurons also are present In the nucleus accumbens, cell clusters as well as a mosaic-like distribution of receptors, transmitters and fibers have been described (see Groenewegen et al , 1991 and see below) Cells within the accumbens can exhibit both spontaneous and evoked electrophysiological responses Evoked responses may appear in, e g , reaction to stimulation of hippocampal inputs (Hakan et al , 1989, Boeijinga et al , 1990)

Glutamatergic hippocampal and dopaminergic mesolimbic afferents may converge on the dendrites of the same neuronal targets (Sesack & Pickel, 1990, Totterdell & Smith, 1989), making postsynaptic interaction possible Furthermore, a presynaptic modulation of glutamatergic transmitter release by dopamine may exist (Yang & Mogenson, 1986) The reverse, a glutamatergic modulation of dopamine release, also has been reported (Imperato, Honoré, & Jensen, 1990)

Nucleus accumbens efferents project to motor areas, like the ventral pallidum and subpallidal areas, entopeduncular nucleus and the mesencephalic reticular formation (figure 3, Groenewegen and Russchen, 1984, Mogenson et al , 1983) The ventral pallidum is known to project a o via the subthalamic nucleus to the thalamus, while this latter structure sends efferents to (frontal) cortical areas Together, these connections represent a parallel organization of many (sub)cortico-striato-pallidal-thalamo-cortical loops (see Groenewegen et al , 1991)

A feedback-loop, from the accumbens back to the VTA, is found to exist However, the main projection from the accumbens to the mesencephalic dopamine cells is to the substantia nigra, both to the A9 cell-group in the pars compacta as well as to the pars reticulata (Groenewegen et al , 1991, Heimer et al , 1991) As the substantia nigra innervates the dorsal striatum, the ventral striatum thus appears to be capable of influencing the dopaminergic input to the dorsal striatum

In addition, modest projections from the nucleus accumbens are also directed to limbic areas, such as the lateral septum, the amygdala and the bed nucleus of the stria terminalis (see Pennartz, 1992, Heimer et al , 1991, Sesack and Pickel, 1990) Projection neurons from the accumbens are found to be mainly GABAergic and peptidergic

Dorsal striatum

The dorsal striatum receives an important dopaminergic innervation from the A9 dopamine cell-group in the pars compacta of the substantia nigra (Snpc, see figure 3) The substantia nigra is located lateral to the VTA (Ungerstedt, 1971) Dendritic fibers from neurons in the ventral part of the SNpc extend ventrally into the cell-poor pars reticulata of the substantia nigra (SNpr) (Gerfen et al , 1987)

A large amount of input comes from the entire neocortex, from sensorimotor cortices as well as associative cortical areas (figure 3, see Vrijmoed-De Vries, 1985, Graybiel and Ragsdale, 1979) These cortical projections appear to be excitatory and to use glutamatergic

neurotransmitters (Walaas, 1981, Fonnum et al , 1981) Furthermore, afferents arrive from the intralaminar thalamic nuclei (Veening et al , 1980)

The ventral part of the dorsal striatum also receives afferents from limbic (hippocampal) areas (Heimer et al , 1982)

Cytoarchitectonically, the dorsal striatum looks homogeneous the medium-sized spiny projection neurons form over 90% of the total cell population in the dorsal striatum (Kemp and Powell, 1971, Smith and Bolam, 1990) Most of them contain the neurotransmitter GABA and a large portion also use either substance P or enkephalin (Gerfen and Young, 1988)

However, similar to the nucleus accumbens, a neurochemical compartmental organization can be found in the dorsal striatum, which is briefly described below

Efferents from the dorsal striatum are directed to the globus pallidus and the substantia nigra, pars reticulata in a highly ordered manner (figure 3, Parent, 1986) Within the substantia nigra they appear to terminate on both dopaminergic and GABAergic output neurons (Gerfen et al, 1987)

As in the ventral striatum, the actual existence of many parallel circuits has been discovered, which originate from all over the neocortex and project via the striatum, pallidum, subthalamic nuclei and the thalamus back to the cortical areas Also direct connections between the striatum and the thalamic nucleus have been found (Alexander and Crutcher, 1990) Contrary to previous views on a convergence of cortical information in the striatum, the existence of such parallel circuits implies that information from different cortical areas is processed separately in segregated neural loops

Compartmental organization

Both the dorsal and ventral striatum display a mosaic-like (or compartmental) organization of fibers, neurons, receptors and/or neurochemical substances

The nucleus accumbens is cytoarchitectonically and neurochemically inhomogeneous Clusters of small cells are found in the medial and ventral parts of the accumbens A so-called core and shell region can be distinguished, more or less corresponding with the distribution of the cell clusters The shell contains cell-poor regions with scattered neurons and clusters of cells at its dorsal and lateral borders, that are dispersing ventrally into the shell The central core contains more closely packed and more homogeneously distributed neurons (Groenewegen et al , 1991, Herkenham et al , 1984)

A differential localization of several receptors and neurochemicals has been found in the accumbens For instance, regions with low AChE activity (Herkenham et al , 1984) and clear patches with the opioid peptide enkephalin are found (Voorn et al , 1989, Groenewegen et al , 1991)

In addition to the above described topographical organization of the afferents to the accumbens, these fibers may relate to distinct compartments, depending on the site of origin For example, hippocampal inputs originating from the dorsal subiculum terminate rostrally in both shell and core region, inside and outside enkephalin-staining patches In the caudal shell

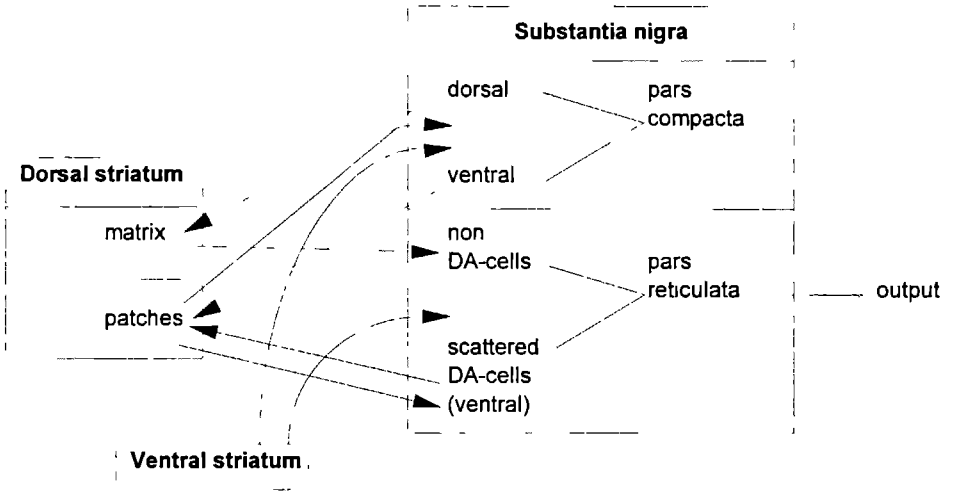

Figure 4. Schematic representation of the connections between the striatal areas and the substantia nigra.

region, fibers from the ventral subiculum preferentially project to the cell-poor area, avoiding the cell clusters (Groenewegen et al., 1987). Dopamine innervation appears to be associated with the caudomedial cell-poor region (Voorn et al., 1986). Also the efferents appear to originate from different compartments within the accumbens (Groenewegen et al., 1991).

The dorsal striatum looks rather homogeneous cytoarchitectonically, but appears heterogeneous in the distribution of fibers and several neuroactive agents when immunohistochemical techniques are employed.

A distinction has been made in so-called striosomes embedded in a matrix, based on the staining pattern of the enzyme acetylcholinesterase (AChE) (Graybiel and Ragsdale, 1983). The distribution of other neuroactive substances and of several types of receptors (e.g. enkephalin, somatostatin, substance P and the opioid, cholinergic and dopaminergic receptor) also shows a heterogeneous pattern, that corresponds more or less to the above mentioned distribution in AChE-poor striosomes and an AChE-rich matrix. Such a compartmentalization can be found in the dorsal striatum (nucleus caudatus) of several species (see Graybiel et al., 1981; Gerfen, 1985; Nastuk and Graybiel, 1988).

Both dopaminergic and cortical afferents have been found to terminate in so-called islands or patches (Olson et al., 1972; Goldman and Nauta, 1977). In subsequent studies specific relations between the termination pattern of corticostriatal fibers and the diverse striatal compartments have been demonstrated, depending on the (layer of the) cortical area of origin, the striatal region and the animal species involved (Gerfen, 1989; Gerfen, 1992).

Nigrostriatal connections are also compartmentally organized. For example, dopaminergic cells in the dorsal part of the SN**pc** (A9) have been shown to project to the striatal matrix, whereas projections from DA neurons in the ventral SN**pc** and from scattered DA cells within the ventral SN**pr** have been found to terminate in the striatal patches (Gerfen et al., 1987;

Gerfen, 1988, Gerfen, 1992) Reversely, neurons in the matrix return projections to (non-dopaminergic) cells in the SNpr, whereas neurons in the patches send axons to the DA cells in the SNpc and ventral SNpr (Gerfen, 1985, Gerfen et al, 1987, see figure 4)
In conclusion, diverse patterns of connections, neurons and (transmitter-)activity are present in both striatal areas As yet, there is no consensus as whether the structure of the striatum is basically bicompartmental or more complex (see Groenewegen et al , 1991)

Dopamine receptor-systems

Several dopamine receptor subsystems can be distinguished
On the basis of electrophysiological and behavioural studies a distinction between the so-called DA_e and DA_i receptors has been made (Cools and van Rossum, 1980) Stimulation of the former kind (by means of dopamine or apomorphine or the indirect acting amphetamine) leads to excitation of neuronal activity and to a short-term increase in locomotor activity in the rat Haloperidol antagonizes this receptor subtype Stimulation of the latter (with dopamine or DPI (3,4-dihydroxy-phenylamino-2-imidazoline) leads to inhibition of neuronal activity and to a long-term suppression of locomotor activity in rats The DA_i receptor can be inhibited by means of ergometrine, which produces its effect only after a latency of 30 to 60 minutes The DA_e- and DA_i-receptors appear to be primarily present within the dorsal and the ventral striatum, respectively (Cools and van Rossum, 1980)
A second concept on dopaminergic receptor subtypes has emerged from biochemical analyses Some receptors were found to be positively coupled to a cAMP generating system the so-called D1 receptor Others were negatively or not coupled the D2 receptor (Stoof and Kebabian, 1984) Recently, in molecular biological studies a dopamine D3, D4 and D5 receptor have been characterized, apparently constituting a D1-like receptor subgroup (D1, D3, D5) and a D2-like receptor subgroup (D2, D4) (Seeman and Grigoriadis, 19987, Tol et al , 1991, Sokoloff et al , 1990) Several selective agents for D1 and D2 receptors have been developed, such as the D1 agonist SKF 38393, the D1 antagonist SCH 23390, the D2 agonist LY 171555 and the D2 antagonists sulpiride and raclopride Both D1 and D2 receptors have been found in both striatal areas (White and Wang, 1986, Richfield et al , 1989) and have been related to a diversity of behaviours (Beninger et al , 1989, Bordi and Meller, 1989, Fletcher and Starr, 1987, Spealman et al , 1992)

1.4 A DIFFERENTIAL ROLE FOR DOPAMINE IN THE VENTRAL AND DORSAL STRIATUM IN BEHAVIOUR

Dopamine has been implicated in a diversity of behaviours, such as locomotor behaviour, sniffing, rearing and stereotypies (Ungerstedt, 1979, Costall and Naylor, 1979, Beninger, 1983, Bos et al , 1988, Kuczenski et al , 1991, Yim and Mogenson, 1989), feeding and sexual behaviour (Pfaus and Phillips, 1989, see review of Blackburn et al , 1992) Especially its role in locomotor and general motor activity is well-known In general, stimulating the dopaminergic activity induces enhanced locomotion or stereotypy, while inhibition of the dopaminergic

activity, e g by means of application of the neuroleptic um haloperidol (DA antagonist), results in reduction of locomotor activity and sedation (see Beninger, 1983, Ploeger et al , 1992, Ungerstedt, 1979)

Externally versus internally directed behaviour

Recently, Oades (1985) proposed a general working principle for the action of dopamine Reviewing a large body of literature he hypothesized that increasing the activity of dopamine in a given dopaminergic brain region promotes the likelihood of switching between alternative sources of information to that region As a consequence of this switching, new input may get (higher) priority over the ongoing input (see figure 5) On a behavioural level the effect is likely to be seen in a change in the temporal patterning of a response sequence or in the initiation of new responses Thus, dopamine appears to be involved in allowing an animal to switch its ongoing behaviour

Studies from our laboratory have established a differential role for dopamine in the two main dopaminergic brain areas, the ventral and dorsal striatum, with respect to its role in behavioural switching Dopamine in the dorsal striatum allows an animal to switch its behaviour arbitrarily, i e enhanced dorsal striatal dopaminergic activity enables the animal to switch its ongoing behaviour without the help of currently available sensory information or stimuli (see review of Cools et al , 1984) For example, the dopamine agonist apomorphine injected into the dorsal striatum enhanced the ability of rats to select the best (life-saving) strategy to cope with the stressfull situation of a one-trial forced-swimming test (Cools, 1980), without being directed by external stimuli Injection of the antagonist haloperidol in the dorsal striatum in cats, trained to walk on a treadmill, decreased the number of motor patterns that were not directed by exteroceptive stimuli, while switching to exteroceptively directed behaviours was not reduced (Jaspers et al , 1984)

In contrast, dopamine in the ventral striatum is involved in the display of cue-directed behavioural items Enhanced ventral striatal dopaminergic activity enables the animal to switch its ongoing behaviour with the help of external (exteroceptive) stimuli For example, the indirect dopamine agonist amphetamine and the selective D2 agonist LY 171555 increased the number of different cue-directed behaviours in the above mentioned forced-swimming

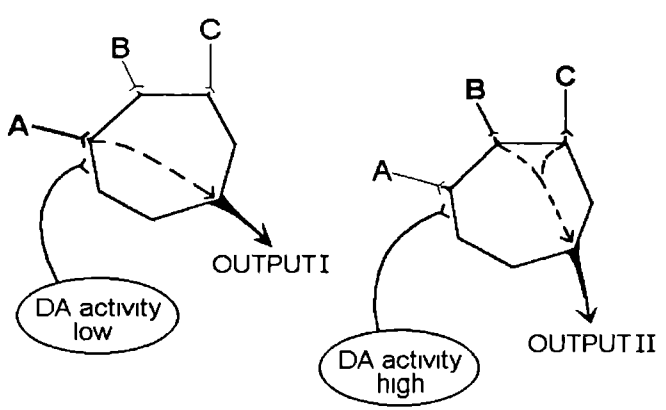

Figure 5 Proposed working principle for the action of DA Increase of DA activity may lead to switching to alternative source of information Adapted from Oades (1985)

test, without affecting the number of different non-cue directed behavioural items (Bos and Cools, 1989, Bos, 1991, Bos et al , 1991)
Overall, these differential roles for ventral and dorsal striatal dopamine have been shown to apply to behaviours in social situations and motor tasks, in rats, cats and monkeys (and for several neurotransmitter-systems) (Cools, 1980, Vrijmoed-De Vries and Cools, 1985, Vrijmoed-De Vries and Cools, 1986, Jaspers et al , 1984, Jaspers et al , 1990, Bercken and Cools, 1982)

Externally versus internally structured learning and memory strategies

In § 2, we have seen that (striatal) dopamine affects many diverse learning and memory processes and the question arose whether some common principle rules this involvement
Clinical and experimental data have implicated the basal ganglia in procedural or implicit memory (characterized by unconscious recollection) (Phillips and Carr, 1987, Mishkin et al , 1984), as contrasted to declarative or explicit memory (characterized by conscious recollection) that is sustained by the frontal and medial temporal lobe (Squire, 1986, Squire, 1992) However, close inspection of data concerning memory impairments in patients suffering from basal ganglia disorders (e g Parkinson's disease) have shown this distinction to be insufficient, such patients may, for instance, show deficits in explicit memory (see Buytenhuijs et al , 1994)
Salamone (1992) and Blackburn (Blackburn et al , 1992) hypothesized that telencephalic dopamine potentiates the ability of significant stimuli to induce the execution of (complex) motor responses in instrumental behaviour
Following our hypothesis on a differential role for ventral versus dorsal striatal dopaminergic activity in motor and social behaviour, we further hypothesize a similar differential role for dopamine in the ventral and dorsal striatum in learning and memory Thus, dopamine in the ventral striatum may be involved in learning and memory retrieval processes directed by external cues, whereas dopamine in the dorsal striatum may play a role in learning and memory retrieval processes that are not directed by external cues (internally directed or arbitrarily)
Free recall versus recognition in humans constitute examples of this distinction between a memory retrieval strategy that is nòt directed by externally available cues and a memory retrieval strategy that is guided by external cues Literature on learning and memory deficits in Parkinson's patients (suffering from brain damage in the nigro-dorsal striatal axis) fairly consistently point to an impaired recall ability, in combination with intact recognition (see Buytenhuijs et al , 1994)

1.5 EMPLOYED LEARNING AND MEMORY TASKS

We have chosen to examine a possible differential involvement of ventral and dorsal striatum in the following three animal tasks These experimental tests offer the possibility to consider

the involvement of ventral and dorsal striatal dopamine in specific cue- or non cue-directed aspects in learning and memory

Social recognition

Social memory concerns information on individual members of a group of organisms, that is important for recognition of and communication among each other This information may be of diverse nature, e g visual, auditory, tactile or olfactory

In groups of rodents, olfactory messages play an important role For instance, rats of one social group excrete odors (so-called odor signatures), discriminating themselves from other groups (Carr et al , 1976, see Popik, 1991) Within groups, odors may involve further individual information on age, sex, position in the social hierarchy and emotional state (alarm pheromones) (see Popik, 1991)

Two subcircuits for processing of olfactory information are recognized (Switzer et al , 1985) First, the *main olfactory system* involves the pathway from the nasal olfactory receptors, via the main olfactory bulb, through the lateral olfactory tract to the piriform cortex (PCx) The PCx (as the main part of the olfactory cortex) further receives inputs from neocortical areas, the basal forebrain, and from the hippocampus and amygdala Output fibers from the PCx run to areas like the neocortex, thalamus and hypothalamus, the basal ganglia (especially the ventral striatum) and the hippocampus This latter area also receives direct input from the olfactory bulb (Hunter and Murray, 1989) In addition, the so-called 'olfactory amygdala' (comprizing a o the nucleus of the lateral olfactory tract, the anterior amygdaloid area and the amygdalopiriform transition area) receives innervation directly from the main olfactory bulb as well as from the piriform cortex (Haberly and Bower, 1989, Olmos et al , 1985)

The second circuit is the *accessory olfactory system*, starting at the chemosensory epithelium in the vomeronasal organ in the nasal cavity Fibers project to the accessory olfactory bulb, which in turn innervates the medial amygdaloid group ('vomeronasal amygdala', including the bed nucleus of the accessory olfactory tract, the medial amygdala and the posteromedial cortical nucleus) (Olmos et al , 1985, Switzer et al , 1985) This latter circuit may be important for social recognition, as it has been reported that the vomeronasal olfactory circuit is involved in the action of pheromones (Segovia and Guillamón, 1993)

Olfactory learning and memory (of social as well as non-social odors) can be tested in several kinds of experiments, like the habituation-discrimination test (Hunter and Murray, 1989, see Popik, 1991) or the positive reinforcement experiment (see Popik, 1991)

Odor-based social memory can be studied by exposing adult rats to juvenile conspecifics (thus avoiding the occurrence of aggressive and sexual behaviours) (Thor and Holloway, 1982) We have employed this paradigm, in which social recognition (SR) is defined as the decrease in social investigation time (SIT, in particular anogenital sniffing) on repeated exposure of the tested adult rat towards the juvenile A decrease in SIT only occurs at short inter-trial intervals (ITI) of about 40 minutes or less (Popik, 1991, Sekiguchi et al , 1991), suggesting that it concerns a short-term, capacity-limited kind of memory A decrease in SIT does not occur

when a novel juvenile is presented at the second exposure (Thor and Holloway, 1982) Recognition appears to be based on olfactory cues emitted by the juvenile (Sawyer et al , 1984, Popik, 1991) Agents as neurohypophyseal peptides (Popik, 1991, Dantzer et al , 1987) and nootropics (Perio et al , 1989) have been reported to specifically affect SR Furthermore, the septum and the medial pre-optic area have been found to play a role in SR (Popik, 1991) In contrast, systemic amphetamine was not specifically effective (Perio et al , 1989)

Morris water maze

The Morris water maze procedure has been developed by Richard Morris (Morris, 1981, Morris, 1984), as a tool to study spatial learning and memory

In its standard version, the animal is required to locate a hidden platform to escape from a large swimming pool, which is rapidly learned during a series of training trials

The ability of localizing an undetectable platform is assumed to depend on the animal's capacity of constructing a spatial map of the environment based on the relations among stimuli or cues, from outside the pool (extra-maze or distal cues) (Morris, 1981) It constitutes an example of O'Keefe and Nadels locale strategy (1978) Spatial memory in the rat is shown to depend on the extent of the environment the animal can see and the length of time it is allowed to look (Mazmanian and Roberts, 1983)

Retention of the location of the platform may be measured in a probe trial (platform removed from the pool), by the time spent in a small region (quadrant) enclosing the former location of the platform Furthermore, the platform may be repositioned, thus examining an animal's ability to abandon the previous learned response and acquire a new one

In contrast, the platform may be 'cued' it may protrude just above the water surface, being clearly visible to the rat from all places within the pool and acting as an intra-maze, proximal cue (a clear beacon) This task demonstrates non-spatial escape learning and controls for sufficient sensorimotor capacities and motivation of treated animals (Morris, 1981, 1984)

Many studies have provided evidence that the hippocampus is particularly important for spatial learning and memory in the Morris water maze (Morris et al , 1982, Sutherland and Rodriguez, 1989, see Brandeis et al , 1989) Acquisition of the cued, non-spatial response was not affected by hippocampal treatments (Morris et al , 1982) Furthermore, the (medial) frontal cortex and the anterior thalamic area have been implicated in standard Morris maze learning (Sutherland et al , 1982, Sutherland and Rodriguez, 1989) Manipulations of catecholaminergic and cholinergic activity have also been found effective (Whishaw and Dunnett, 1985, Hagan et al , 1983, Lindner and Schallert, 1988, Whishaw, 1989)

Radial arm maze

Radial arm mazes (RAM) can be employed to study spatial versus non-spatial learning (Mazmanian and Roberts, 1983, Toumane et al , 1988) as well as working versus reference memory (Knowlton et al , 1985, Buresová and Bures, 1982)

A varying number of arms can be used (4, 8 and 17 arm-mazes are common), radiating from a central platform

In its most simple version, an animal is required to collect one food-pellet from the end of each arm in an efficient way (i e without revisits) With the maze in a constant position and not covered to hide the environment, the animal is able to rely on extra-maze cues and spatial mapping abilities to solve the task

When some of the arms are never baited, the animal needs to learn this trial-independent information (RM) Information on which of the baited arms has been visited within one trial refers to WM

Furthermore, animals may acquire fixed and quick responses when collecting all the pellets, based on the use of egocentric localization (orienting from the animals own body axis)

The hippocampus, the medial septal area and the nucleus basalis magnocellularis appear to play a role in the working memory component (Olton et al , 1979, Knowlton et al , 1985) Lesions of the mediodorsal thalamic nucleus impaired both RM and WM (Stokes and Best, 1990) Furthermore, cholinergic and dopaminergic activity have been shown to be involved in RAM behaviour (Mundy and Iwamoto, 1988, McGurk et al , 1989)

We have employed a simple radial arm maze task, involving the collection of one food pellet from each of four radiating arms A clear stimulus was attached on the wall near each of the arms In this maze, behaviour includes externally guided aspects (for instance, remembering which arm already has been visited in relation to its nearby stimulus) as well as arbitrarily directed aspects (for instance, starting to visit arms or developing a fixed response pattern)

1.6 Aim and outline of the present thesis

A differential role for ventral versus dorsal striatal dopamine in behaviour has been put forward Dopaminergic activity in the former area has been linked to switching of behaviour directed by external stimuli or cues, whereas dopaminergic activity in the latter areas has been associated with arbitrarily directed behaviour, not guided by external cues

Dopamine has been shown to affect learning and memory Its role has been related to the potentiation of the ability of significant stimuli to induce motor responses Others have implied a role for the basal ganglia in implicit or procedural learning and memory Based on previous work from our laboratory, a differential role for the dopaminergic activity in the ventral versus the dorsal striatum in learning and memory is proposed the ventral striatum may be involved in learning and memory processes that are directed by external cues, whereas the dorsal striatum may play a role in learning and memory processes that are arbitrarily guided

This thesis examines the hypothesis of that differential role of striatal dopamine in three different learning and memory tasks These three tasks are suitable for studying the effects of dopaminergic treatment in the ventral and dorsal striatum on cue-directed and non-cue directed aspects of learning and memory retrieval strategies

In chapter 2, the effects of post-training manipulation of the dopaminergic activity at the level of the DA_1 receptor in the nucleus accumbens (ventral striatum) are studied in the social recognition task

Chapters 3 to 5 describe studies on spatial learning in the Morris water maze First, the effects of systemic injections of a dopaminergic antagonist on spatial learning are regarded Second, it is investigated which of the striatal areas participate in the observed effects

In chapters 6 and 7, radial arm maze behaviour is examined Both the involvement of dopamine in retention of a previously acquired maze response and the involvement of dorsal striatal dopamine in radial arm maze acquisition are considered

Chapter 8 offers a general discussion of the main results and their interpretation A summary is finally presented with the main conclusions from each chapter.

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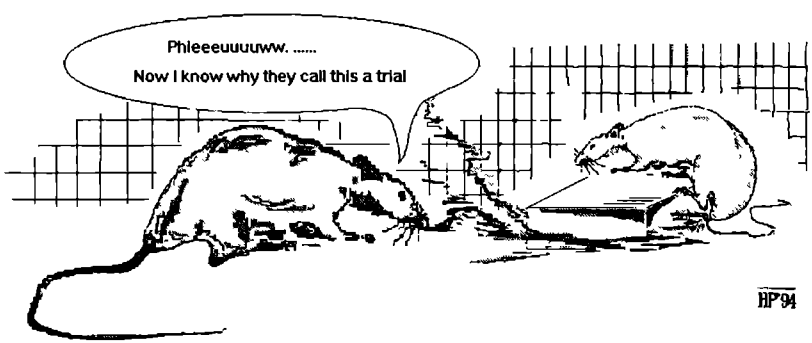
ANIMAL RESEARCH
PART I: SOCIAL MEMORY



CHAPTER 2.

ROLE OF THE NUCLEUS ACCUMBENS IN SOCIAL MEMORY IN RATS

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ABSTRACT

Recognition of a juvenile conspecific by an adult male rat is known to be reflected by reduced anogenital investigation (A G I) of this young individual by the adult, when the two animals are re-exposed to each other after some short delay As the delay is increased, the reduction in A G I is reduced This measure of social memory can be modulated by several drugs, among others cholinomimetic agents In this study, the effects of direct manipulation of the nucleus accumbens were studied Local administration of (3,4-dihydroxyphenylimino)-2-imidazoline (DPI 0 1-1 5 μg) decreased investigatory behaviour at the second exposure after a long interexposure-interval, while ergometrine (0 1 μg) counteracted this reduction by DPI These findings suggest a role for the nucleus accumbens in social recognition, in particular for the so-called DA1 receptors which are stimulated and inhibited by DPI and ergometrine respectively

INTRODUCTION

Adult male rats appear to be able to form a so-called social memory for juvenile conspecifics (17) Normally, an adult rat thoroughly investigates a novel young rat when exposed to it When re-exposed to this juvenile after a short interval, the adult rat shows much less investigatory behaviour With longer intervals, recognition vanishes and the juvenile is again thoroughly investigated (8,10)

This social memory is based upon olfactory cues emitted by the stimulus animal (2,15), it is sensitive to retro-active facilitation and interference (8), and can be modulated by several drugs Peptides like vasopressin (8,10) as well as cholinomimetics, nootropic drugs and benzodiazepine inverse agonists (14) can influence the duration of investigation of the same young stimulus animal, while not affecting the investigation of a different young rat

In studies on drug-induced changes in social memory, the drugs were applied either systemically or intracerebro-ventricularly So, little is known about the underlying mechanisms or brain regions involved in these memory processes In this study we investigated whether the nucleus accumbens plays a role in this kind of memory, for reasons outlined below

First, the nucleus accumbens is known to link the hippocampus with the ventral pallidum/substantia innominata-complex (19), viz regions which are known to be involved in different kinds of memory (9,11,16) Since, in addition, both structures are cholinoceptive, it is relevant to note that cholinomimetics can modulate social memory (14) Second, recent studies from our laboratory have shown that the nucleus accumbens is involved in switching behaviour with the help of available external cues (1) So, the question arose whether the nucleus accumbens also allows the organism to use cue-directed strategies to search for stored information In other words is the nucleus accumbens also involved in memory processes, in which recognition of some object takes place on basis of external cues? Therefore, we studied the effects of direct manipulation of the dopaminergic activity in the nucleus accumbens on social memory in rats

We choose to use (3,4-dihydroxyphenylimino)-2-imidazoline (DPI) and ergometrine, because these agents have been found to be behaviourally effective in a highly specific manner when injected into the nucleus accumbens of well-habituated and well-handled rats (7) DPI and ergometrine are considered to be an agonist and an antagonist of the so-called DA1 receptors respectively Activation of the DA1 receptors in the nucleus accumbens by dopamine or DPI results in a long-term suppression of the locomotor activity in well-habituated rats, whereas an injection of ergometrine into the nucleus accumbens increases the locomotor activity in such rats This effect of ergometrine can be counteracted by DPI and dopamine, while noradrenergic agents are ineffective in this respect (see for more details ref 3, 5 and 6) Since neither intra-accumbens injections of selective D1 agents (SKF 38393 D1 agonist, SCH 23390 D1 antagonist) nor such injections of selective D2 agents (LY 171555 D2 agonist, l-sulpiride D2 antagonist) have been found to produce such changes (7), it was decided to disregard the use of these drugs, despite their known specificity as far as it concerns D1 and D2 receptors respectively

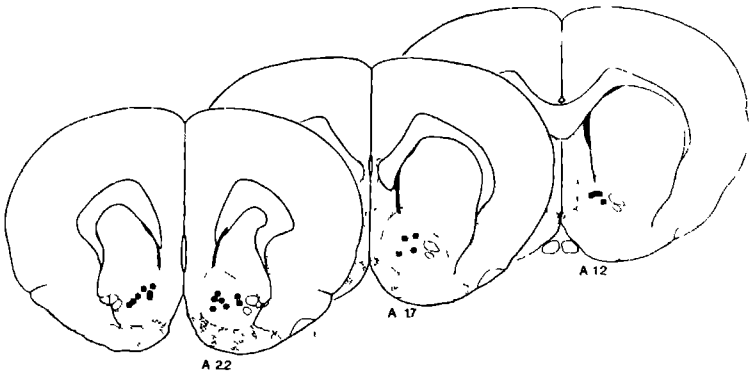

Figure 1. Representative series of injection sites in the nucleus accumbens. The planes are taken from the atlas of Paxinos and Watson (1982), the A-coordinate is in mm from bregma.

MATERIAL AND METHODS

Animals and surgery

The subjects were male Wistar rats, formerly used as breeders and about one year old (weight: 400-600 g). After the last time they have been used for breeding and just prior to the experiments, they were held together with the female and the newborn rats. So, they were familiar with juvenile rats.

Thereafter, upon arrival at our laboratory, they were housed individually and kept at a reversed light/dark cycle (lights on at 19 00 h; lights off at 7.00 h). Water and food were available ad libitum.

Per experiment 12 exbreeders and 72 juvenile conspecifics (24-27 days of age; housed in groups of six, in the same animal room) were used.

For implantation of the cannulae the adult rats were anaesthetized with pentobarbital (Narcovet®; 60 mg/kg i.p.) and placed in a stereotaxic apparatus. Stainless steel cannulae (length: 6 mm; diameter. 0.5 mm) were bilaterally implanted, aiming at the nucleus accumbens (coordinates: A=bregma + 1.85, L=+/- 1.2, H=2.7 mm, based on the atlas of Paxinos and Watson (13)). The cannulae were brought in with a lateral angle of 10° and fixed onto the skull with dental acrylic cement (Paladur®) and stainless steel retaining screws (1).

After surgery, the animals were allowed two weeks of recovery. On three days during the second week of recovery, each rat was handled for a few min between 10.00 h and 15.00 h. Then, after the recovery period, the experimental procedure started.

Experimental procedure

For each group the following experimental procedure was carried out. First, each single adult rat was exposed twice (each time for 5 min) on one day to the same juvenile; this was done four times on four successive days (day 1-4) with increasing inter-exposure intervals (5, 10, 30

and 120 min) and with a new juvenile for each new interval Thus, it could be established whether they did show recognition for a young rat at short intervals and whether recognition was reduced at a longer interval Both recognition at a short delay and decreased recognition after a longer delay were prerequisites for continuing the experiment During these days they were also handled, and they received a sham injection on the fourth day So, the rats were well habituated to being handled as well as to the injection-procedure (see below)

Next, the adult rats were again tested on day 9 and day 11 at an inter-exposure interval of 120 min On day 9 the animals received a control injection of the solvent (AD) in order to establish the baseline (see below), while an injection of a dopaminergic agent, viz DPI and ergometrine, was given on day 11

Injections were given bilaterally, using a Hamilton syringe, with the needle extending 1 5 mm below the end of the cannula, thus reaching the nucleus accumbens The injected volume of 0 5 µl per side was delivered over a period of 5 s, thereafter the injection needle was kept in place for another 5 s

DPI and ergometrine were dissolved in distilled water (AD), per experimental group, one of the following doses was used on day 11 0, 0 1 and 0 5 µg for DPI, 0 1 µg for ergometrine, or DPI, 0 5 µg, in combination with ergometrine, 0 1 µg Apart from ergometrine, all injections on day 9 and 11 were given immediately after the first exposure Ergometrine was administered 1 h before DPI on day 11, this was done, because the inhibitory action of ergometrine at the level of the DA1 receptor is known to start after a delay of 45 to 60 min (4)

Histological verification

After the experiments the animals were sacrificed by an overdose of pentobarbital, the brains removed and fixated with 4% formaline, and the precise localization of the cannulae and injection sites were determined in serial sections using cresyl violet staining Only the animals with correctly placed injections were included in the statistical analysis

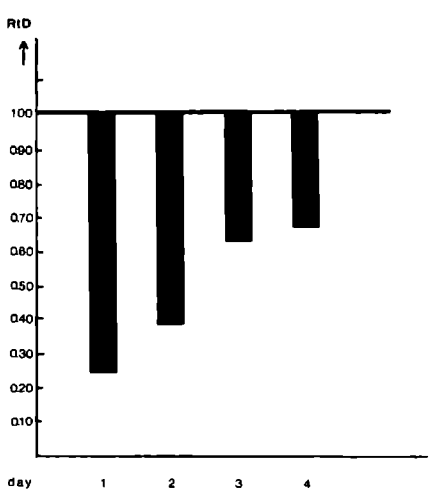

Figure 2. Ratios of investigation duration (RID = duration of second exposure/ duration of first exposure black columns) for day 1-4 with increasing interexposure intervals from one experiment (n=10) Shown are median values The solid line represents performance during the first exposure (100% = ratio 1)

Table 1 Raw data for the duration of investigation during the first and second exposure on four successive days with increasing inter-exposure intervals from one typical experiment Investigation at the second exposure was significantly shorter than at the first exposure after short intervals (5-30 min) while such a reduction was not anymore present at a longer interval (2h), shown are median values Note the same juvenile was presented during the first and second exposure on one day, while different juveniles were presented on different days

	DURATION OF INVESTIGATION			
	INTER-EXPOSURE INTERVAL (min) ON DAY 1-4			
DAY	1	2	3	4
EXPOSURE INTERVAL	5	10	30	120
1st median	58 8	45 5	38 5	48 8
'range[a]	36 9-68 4	36 1-50 9	20 6-65 6	29 8-71 6
2nd median	12 3**	15 5**	26 8*	34 5
'range[a]	8 9-19 9	10 3-27 5	6 0-43 0	17 5-59 9

Significances * $p<0.02$, ** $p<0.01$, Wilcoxon matched-pairs signed-rank test
[a] An indication of the variation is given by the 'range' for each median the 25% and 75% values are given

Behavioural analysis

Social investigation was expressed in terms of duration (seconds) and frequency (number of 'bouts') of anogenital sniffing per exposure Each time that anogenital investigation (A G I) occurred, separated by other behavioural items, this was counted as one 'bout', total duration and total number of A G I were calculated

Per day and for each animal, the ratio of duration (resp frequency) of the second exposure to that of the first exposure was taken This was done in order to minimize (a) inter-individual variations and (b) day-to-day variations in baseline performance (first exposure)

The computed ratio of day 9 (control day, effect of handling and injection) was taken as a baseline and the ratio of day 11 (drug day) was compared to this value the difference between these ratios was regarded as reflecting the effect of the drug itself

Furthermore, the overall behaviour (duration and frequency) during the second exposure was compared between a DPI- and a control-day, using a standard list of behavioural items (18), these were chosen in such a way that occurrence of one item excluded the occurrence of any other one During these two exposures, the adult rats exhibited an equal amount of anogenital investigation, so, it was possible to compare the remainder of the behaviour, shown by the rats in these exposures This analysis was done in order to check whether or not the DPI-induced changes in A G I were accompanied by additional changes in other behavioural items, for example locomotor activity

Statistics

A Wilcoxon matched-pairs signed-rank test was used to test intra-individual differences between first and second exposure, while a Mann-Whitney *U* rank order test was used to test for significant differences between experimental groups (both tests two-tailed) (12)

RESULTS

A representative series of injection sites is shown in figure 1 Per experiment, 1-3 animals had to be discarded because of location of the injection sites outside the nucleus accumbens The remainder of the injections sites was found within the dorso-medial part of the nucleus accumbens (anterior coordinate 1 2-2 2 mm from bregma (13)) See for exact numbers of rats per experiment figure 3

Raw data for the duration of investigation during the first and second exposure at different inter-exposure intervals (on day 1-4) for one typical experiment are shown in table 1 Only the data for the duration are given, since the frequency revealed the same picture

There was no significant difference in anogenital investigation during the first exposures on day 1 to 4 At short intervals (5 to 30 min), duration of investigation at the second exposure was significantly shorter than that of the first exposure (Wilcoxon, $p<0\ 02$ at 30'-interval, $p<0\ 01$ at 5'- or 10'-interval), while such a reduction was not anymore present at a longer interval (2h)

Figure 2 shows the ratios of investigation duration (RID), computed from these raw data Performance during the first exposure is represented by the solid line (100% ratio 1), the columns represent the ratio of investigation duration of the second exposure to the first Plotted are the median values

In figure 3, the ratio of day 11 (drug-injection) minus the ratio of day 9 (injection of solvent, viz the baseline, reflecting the effect of handling and injecting) is shown in order to illustrate the drug-induced effects

DPI reduced the anogenital investigation at the second exposure at a 2h-interval, at a dose of 0 5 µg/0 5 µl This figure also shows that the reduction by DPI could be antagonized by ergometrine at a dose of 0 1 µg/0 5 µl Ergo-metrine, in the dose mentioned, had no effect on its own, neither on the first exposure (not shown), nor on the second exposure (fig 3)

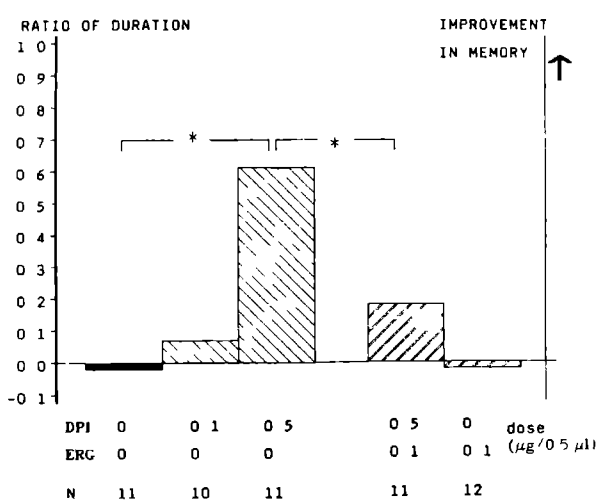

Figure 3. Effect of DPI (0, 0 1, 0 5 µg) and of ergometrine (0 1 µg) on anogenital investigation in rats Along the y-axis the difference between the ratios of duration (second exposure to first exposure) of day 11 (effect of druginjection) and day 9 (effect of control injection and handling), per experiment is plotted (see also text) Shown are median values Significances * $p<0\ 05$, Mann-Whitney *U* test

Table 2 Overall behavioural analysis frequencies and duration as a percentage of total frequenties resp of total observation time for several behavioural parameters during the second exposure of a control-day (no injection, second exposure after 30 minutes, n=12) and of a DPI-day (DPI 0 5 µg/0 5 µl, second exposure after 2h, n=11) Shown are mean values The behavioural items covered for the total observation time (after (18)) These two groups showed the same amount of anogenital investigation Investigation of the juvenile includes both sniffing and grooming of the body of the young rat, but not anogenital investigation! No significant differences were found

BEHAVIOURAL ANALYSIS

	DURATION				FREQUENCY			
BEHAVIOUR	DPI (0 5µg) mean ± sem		CONTROL mean ± sem		DPI (0 5µg) mean ± sem		CONTROL mean ± sem	
ANOGEN INV	13 1	2 4	10 9	1 9	12 8	1 7	10 5	1 2
LOCOMOTION	8 6	1 2	7 5	1 2	18 9	1 8	17 5	2 9
SNIFFING	23 2	3 0	21 7	4 0	18 9	2 0	13 7	2 4
GROOMING	7 2	2 4	5 8	2 1	3 6	0 8	4 5	1 7
BEING SNIFF	6 7	2 9	3 9	1 5	2 3	1 0	4 2	2 2
INV JUV	17 0	3 5	10 4	2 5	13 1	1 9	14 5	2 5
SCANNING	24 0	2 5	39 7	7 2	29 9	1 6	34 9	5 6

A lower dose of DPI did not yet produce an effect (fig 3), whereas higher doses (1 0-1 5 µg, not shown) produced lesser effect, possibly because these doses induced sedation, as has been reported earlier (5)

The overall behavioural analyses (duration and frequency as percentage of total observation time resp of total frequen-cies, see table 2) revealed no statistically significant differences in any of the behavioural parameters, although there was both a slight reduction of scanning and a small increase in the other behaviours in the DPI-group (duration)

Discussion

The present data confirm the results from previous studies that adult male rats are able to recognize juvenile conspecifics This is indicated by reduced anogenital investigation of the juvenile by the adult, during the second exposure after short intervals Though not controlled for in our experiments, Thor and Holloway (17) have shown that such a reduction does not occur when the adult rat is re-exposed to a different juvenile So, it appears that forming a short-lasting memory for an individual juvenile is a specific effect

In contrast to the solvent given on day 11, which did not alter the amount of A G I (fig 3 0 µg DPI), 0 5 µg DPI significantly decreased duration and frequency of social investigation at the second exposure after a long inter-exposure interval (2h) This reflects an improvement in recognition and is taken as an indication that the nucleus accumbens can modulate this social memory

The DPI-induced modulation seemed to be a specific effect of a particular subtype of dopaminergic receptors, the so-called DA1 receptors, within the nucleus accumbens as ergometrine could antagonize the DPI-induced reduction of anogenital investigation Ergometrine had no effect on its own upon the duration of anogenital investigation at the second exposure In addition, while injected 1 h before DPI and thus before the first exposure,

ergometrine also had no effect on the duration of investigation at the first exposure (not shown)

The effect of DPI cannot be attributed to aspecific or indirect effects For, animals showing a DPI-induced decrease in anogenital investigation displayed a behavioural palette, that was more or less similar to that of a group of animals re-exposed to the juvenile after an interval of 30 min and showing a comparable degree of anogenital investigation

Although a more extensive dose-effect analysis has to be made in order to prove that especially DA1 receptors are involved, the present data are sufficient to conclude that the nucleus accumbens plays a role in the noted effects Given the role of the nucleus accumbens in enhancing the ability to switch to cue-directed behaviours (1), it is attractive to postulate that DPI improves social memory, because it facilitates the animal's ability to switch to cue-directed strategies in order to search for stored information This hypothesis needs to be validated in future research It also remains to be investigated whether other neurotransmitters and/or receptor mechanisms within the nucleus accumbens are involved in regulation of social memory

In conclusion, the present results indicate that the nucleus accumbens clearly plays a role in social memory

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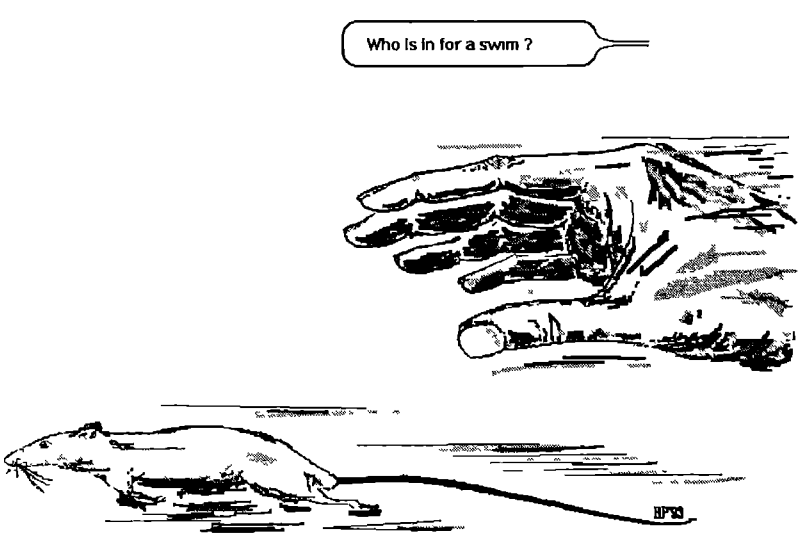
PART II: SPATIAL LOCALIZATION: MORRIS WATER MAZE



CHAPTER 3.
EFFECTS OF HALOPERIDOL ON THE ACQUISITION OF A SPATIAL LEARNING TASK

G.E. Ploeger, B.M. Spruijt and A.R. Cools

Physiology and Behavior 52. 979-983, 1992

ABSTRACT

The effects of systemic injections of the dopaminergic antagonist haloperidol on the acquisition of the Morris water maze with a visible resp invisible platform (non-spatial vs spatial learning) were investigated An open field test was used for selecting a dosage (≤0 1 mg/kg), that would not (or hardly) affect locomotor behaviour Differential effects were found At 0 1 mg/kg, haloperidol both reduced locomotion in the open field and impaired resp blocked acquisition in the Morris maze with a visible resp invisible platform Even though 0 07 mg/kg haloperidol reduced locomotion, both 0 04 and 0 07 mg/kg only impaired Morris maze performance in the spatial version A large effect was found in the first trial of every day's training block These results indicate that haloperidol at low doses can lead to a moderate but significant impairment of spatial learning It is suggested that the effects found are related to the function of the striatal areas in cue- and non cue-directed behaviour

INTRODUCTION

Dopamine (DA) is known to be involved in various kinds of behaviour Most prominent is its role in (loco)motor behaviour [2, 10, 11, 14] Dopamine has also been implicated in more cognitive functions, among which learning and memory [2, 16, 19] For example, it has been demonstrated that amphetamine (an indirect DA-agonist) has memory-improving effects [17, 18], neuroleptics, as potent dopamine-antagonists, strongly disrupt the acquisition of conditioned avoidance responding [5], and there is good evidence that DA plays a role in reward-related incentive learning [2, 3]

Animal studies from our laboratory have investigated the possible involvement of dopamine in allowing an animal to switch behaviour A differential role has been established for the two main dopaminergic structures, i e the neo- or dorsal striatum and the nucleus accumbens (ventral striatum) Neostriatal dopaminergic activity appears to affect switching of behaviour directed by factors intrinsic to the animal (non cue-directed or arbitrarily) This is shown for both motor and social behaviour, in rats and cats as well as in monkeys [4, 9, 25, 26, 27] Mesolimbic dopamine affects switching motor behaviour aided by external available cues (cue-directed) [6, 7, 8]

It is hypothesized that this differential function of dopamine via specific brain areas underlies the efficacy of dopaminergic agents to affect different learning tasks

For several reasons we choose to use the Morris water maze to study the dopaminergic influence on cue-directed learning First, this is a task in which animals heavily depend upon external (distal) cues in learning to locate a hidden platform (spatial learning) Second, there is evidence that the dopaminergic striatal areas are linked to brain areas, like the hippocampus and the ventral pallidum/substantia innominata-complex [30], that are implicated in mnemonic processes, among which spatial learning in the Morris watermaze [12, 13, 15, 23] Third, several reports (which either used a lesioning technique or rather high doses of dopaminergic agents) have already pointed out that dopamine and the dopaminergic brain areas mentioned above, play a role in the acquisition and retention of the watermaze task ([1, 28, 29], see discussion)

In those studies however, it was not clearly shown that the applied dopaminergic manipulations specifically affected the behaviour at the cognitive level This study investigated the effects of low doses of the potent dopaminergic antagonist haloperidol (systemically injected, to start with), in the training phase of the Morris maze task The performance of rats both in the spatial task with a hidden platform and in a version with a visible one (to check for sensorimotor abilities) was compared The dosage of haloperidol used was based upon previous experiments from our laboratory [9] and tested in a simple open field test in order to avoid difficulties in the interpretation of the results because of the possible occurrence of motor disturbances

Animals

Male Wistar rats, weighing 250-300 g at the time of testing, were housed in groups of 3 and kept in a temperature and light-controlled room at a reversed light/dark cycle (lights on between 20 00 and 08 00 h), all experiments were carried out between 09 00 and 17 00 h Water and food were available ad lib

Per experiment a group of 8 animals was used

Haloperidol

The dopaminergic antagonist haloperidol (Janssen Pharmaceutica, The Netherlands), dissolved in saline, was used in the following doses 0, 0 01, 0 04, 0 07 and 0 1 mg/kg It was injected intraperitoneally, 30 min prior to the testing

Experimental tests and procedures

Open field

To test for the effects of haloperidol on locomotor behaviour and exploration, the rats were subjected to an open field test The apparatus consisted of a circular box, 75 cm in diameter and surrounded by a 40 cm high dark wall A 12 cm high steel object was placed in the center of the box

The rats were allowed to move freely and to explore the environment and the object for 10 min The drug (or its solvent) was administered 30 min prior to the test Between injection and the test the animal was brought back into its homecage

For three doses (0, 0 04 and 0 1 mg/kg) the animals were tested in the open field for three consecutive days in order to see whether the animals showed habituation to the drug

During testing the path of each animal was automatically recorded and later analysed (see below) For the analysis three areas were defined one along the edge (border-zone), a second area in which the object was located (object-zone) and a third one in between (rest-zone)

Morris water maze

The apparatus was a black circular pool, 230 cm in diameter and 35 cm deep It was located in a large observation room External cues, that were kept constant, surrounded the pool

The pool was filled with water of 26±1 °C to a depth of 23 cm Behavioural testing was performed under dim red light conditions, with one small light on near the computerized observation system for use of the experimenter

Both a visible white platform, protruding just above the water surface (for the proximal cue task), and an invisible transparent perspex one, hidden below the surface (for the distal cue task), were used The platform, whether visible or invisible, was placed in a constant location

in the center of quadrant 1 Four equally spaced points around the wall of the pool were used as starting points

For each dose one group of rats was trained on the visible task and another on the invisible version Each day the animals were given a block of four trials with an intertrial interval of ± 10 minutes The drug (or its solvent) was injected every day, always 30 min before the start of the first trial Each animal started at a different point each trial The animal was gently placed into the water, facing the wall It was allowed to swim around until it located the platform, or, when it did not find it within 120 s, it was placed on it The rat was left on the platform for 30 s For the visible task, the animals were trained for 3 days, while for the invisible task, the animals were given 4 days of training

In one additional experiment, the effect of removal of some prominent cues from the surroundings on learning the location of a hidden platform was examined and the performance of this group was compared to that of the above mentioned control group on the invisible task that was trained with these cues

As with the open field tests, the path of each animal on each trial was recorded and later analysed (see below)

Behavioural recording and analysis

For both the open field test and the Morris water maze task the path of the animal was automatically registered by a computerized image analysis system The hardware consisted of an IBM AT computer combined with a video digitizer PV VISION PLUS board (Imaging Technology Inc U S A) and a C C D video camera For a detailed description of the software, used for data acquisition and analysis, see Spruijt et al [22]

In short, with a sampling method a picture of the animal was taken and the coordinates of the position of the rat determined These coordinates were then stored into the computer (raw

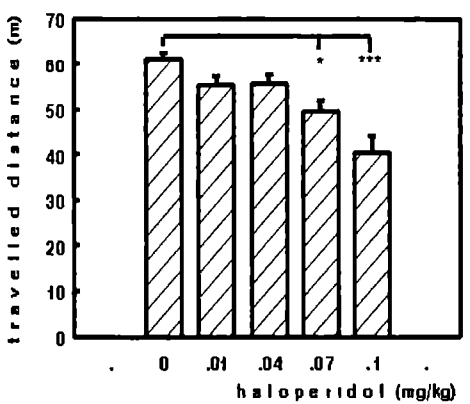


Figure 1.a Total travelled distance (in m) in the open field for increasing doses of haloperidol Shown are mean values±s e m , n= 8 per group Significant differences were found between control (saline) and haloperidol 0 07 and 0 1 mg/kg * p<0 05, *** p<<0 02 (ANOVA and post hoc analysis)

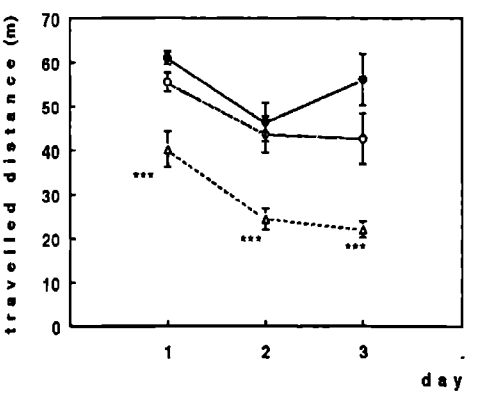


Figure 1.b Mean total travelled distance (in m) in the open field for two doses of haloperidol on three consecutive days (● control, o halopendol 0 04 mg/kg, Δhaloperidol 0 1 mg/kg) The halopendol group of 0 1 mg/kg differed on all three days from the control group *** p<<0 01 (ANOVA and post hoc analysis)

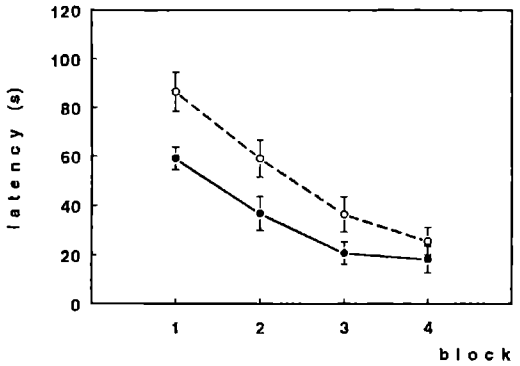


Figure 2 Mean latencies (in s) per group and per block of 4 trials, in the Morris water maze with an invisible platform In one group (›) some prominent cues were removed from the environment during training, whereas in the other group (●) these particular cues were present

data).

Afterwards the raw data were analysed and various computations made So, several parameters, e g latency, travelled distance and time spent within a certain region, were automatically calculated for each animal and per group (means and standard errors) The individual values could then be imported into the statistical package SYSTAT (Wilkinson, Leland, SYSTAT The system for Statistics, Evanston IL SYSTAT, Inc , 1990)

For analysing the effects of the drug an analysis of variance (ANOVA) was used for the effects on one day (open field) For the effects over days (open field and Morris maze) an analysis of variance on one factor for repeated measures was applied Several parameters were regarded (see the results below) The ANOVA for repeated measures was followed by ANOVAs per day, while these were followed by a Tukey HSD procedure for assessing differences between group means per day

RESULTS

Open field

In figure 1 a the total travelled distance per group is shown A significant reduction in travelled distance with increasing doses of haloperidol was observed ($F(4,35)=9\ 8$, $p<<0\ 01$)

Other parameters, like the distribution of time spent and distance travelled over the three areas, or the latency until the animals entered the object zone in the middle of the box, did not show significant changes between any of the groups (not depicted) Also an analysis on the distance travelled per time-period (of 1 min each) within the open field-test (total test time 10 min) did not reveal different activity patterns each group showed the same gradual decline in travelled distance over time within the test session

In panel b of figure 1 the total travelled distance for the three groups, that were tested for three consecutive days, is depicted Analysis of variance revealed an effect between groups ($F(2,18)= 18\ 4$, $p<<0\ 01$) as well as an effect of days ($F(2,36)=19\ 2$, $p<<0\ 01$) No interaction between groups and days was found ($F(4,36)=0\ 19$) Post hoc analysis showed that haloperidol 0 1 mg/kg differed significantly from the other two groups No other parameters revealed important differences between the three groups

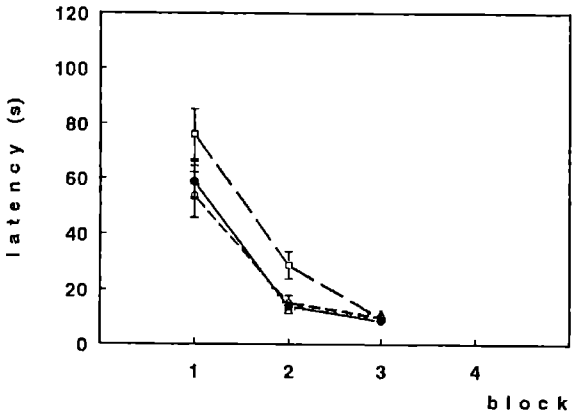
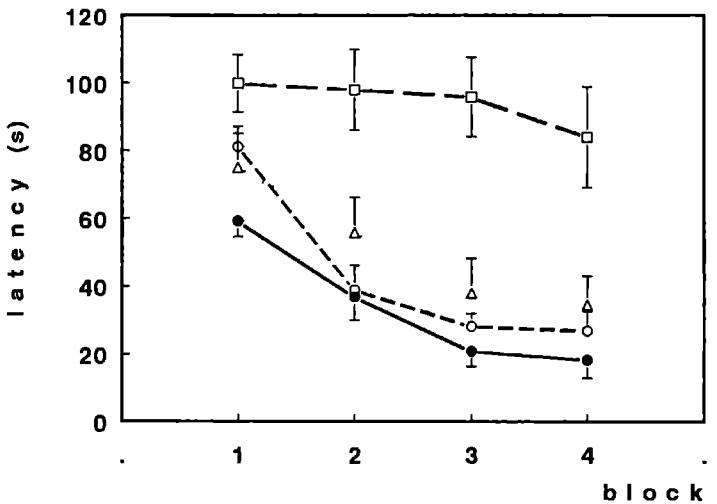


Figure 3.a Latencies (in s) per group and per block of 4 trials in the Morris water maze with a visible platform Shown are mean values ± s e m ● control, O hal 0 04 mg/kg, Δ hal 0 07 mg/kg, □ hal 0 1 mg/kg

Figure 3.b Mean latencies (in s, ± s e m) per group and per block in the Morris water maze with an invisible platform An ANOVA yielded a clear group effect, see text For explanation of the symbols, see figure 3 a



Morris water maze

One group of animals was trained to locate the platform without the use of some prominent cues, normally present in the environment As shown in figure 2, these rats have significantly increased latencies throughout all training blocks as compared to the animals that can use these cues for localization ($F(1,18)=12\ 2$, $p<0\ 01$).

Figure 3 presents the latencies per block for the task with the visible platform as well as with the invisible one As the distance swum gave similar results, this parameter is not shown here In case of a visible platform all groups showed improvement over the days ($F(2,56)=163\ 5$, $p<<0\ 01$) Analysis of variance with repeated measures yielded an overall significant effect between groups ($F(3,28)=2\ 9$, $p=0\ 05$) Posthoc analysis showed that the group of 0 1 mg/kg

haloperidol differed significantly from all three others (p-values ≤0 02) on day 1 and 2 Nonetheless all groups reached the same performance level within the same time

For the task with the invisible platform however, the group with the highest dose of haloperidol hardly showed any improvement over the days (fig 3 b) The overall ANOVA with repeated measures yielded a highly significant effect between groups ($F(3,28)=14\ 4$, $p<<0\ 01$), while also an interaction-effect (group x block) was found ($F(9,84)=2,4$, $p=0\ 01$) A Tukey HSD test revealed that the 0 1 mg/kg haldol-group differed on all days from the three others Examining the performance of this group per trial, they showed a slight improvement only over the first four trials, but from the second day on no further improvement occured The animals from the 0 04 and 0 07 mg/kg haldol-groups remained slower in finding the platform up to day 4 ($F(1,14)=5\ 1$ resp 4 3, $p=0\ 04$ resp 0 057) It is important to note that the effect found is mainly present in the first trial of every new training day Leaving these trials out of the overall ANOVA, no significant differences were found anymore Testing the performance of these groups on every day's first trial, they differed significantly from controls ($F(1,14)=6$, $p= 0\ 03$, see figure 4)

Discussion

When tested in the open field, provided with an object, haloperidol only showed a reduction in travelled distance at a dose of 0 07 and 0 1 mg/kg Other parameters were not affected nor did the doses of 0 01-0 04 mg/kg gave any changes in motor activity in all parameters measured Haloperidol given three times on consecutive days did not induce tolerance to the drug So, we considered this range of doses appropriate in order to dissociate possible cognitive effects from sensorimotor effects of haloperidol in the Morris maze

Performance in the Morris maze with the visible platform revealed that this task is less sensitive to motor disturbances than the open field test haloperidol increased latencies only at the highest dose, on day 1 and 2 The groups of 0 04 and 0 07 mg/kg haloperidol showed excellent performance So, for these last two doses sensorimotor capacities are sufficient

In the task with the invisible platform, it is evident that the animals indeed do depend upon external available cues to direct them to the proper location Considering the effects of haloperidol in spatial localization, a dissociation emerged

Haloperidol at the two lowest doses, while leaving the sensorimotor coordination intact, impaired the acquisition of spatial learning For both dosages there was a large effect on the first trial of every day's new training block, haloperidol 0 07 mg/kg also affected the other trials to some extent So, it appeared that these haloperidol-treated animals have more difficulty in remembering the right location over a long period (1 day) At the highest dose, haloperidol almost completely inhibited the animals from escaping onto the platform This latter dose clearly reduced locomotion in the open field and induced a small but significant deficit in the visible version of the Morris maze, this inhibition therefore can be due to a motor dysfunctioning, superimposed on the learning impairment found at the lower doses Nonetheless, they did learn to switch to energy saving behaviour, meaning that they ceased to

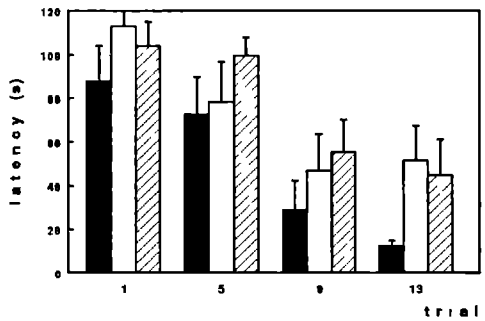


Figure 4. Mean latencies (in s) for every day's first trial (that is for trial 1, 5, 9 and 13) in the Morris water maze with the invisible platform ■ control, □ hal 0 04 mg/kg, ▨ hal 0 07 mg/kg

swim actively, that they sometimes were treading the water or were either floating around in a horizontal position or lying vertically deep into the water At the end of the trial however, they immediately reacted to the approach of the experimenter

So, our results reveal clearly that manipulation with dopaminergic activity by means of low doses of the DA antagonist haloperidol can lead to a moderate but significant impairment in spatial learning and that on top of this cognitive effect, motor disturbances can affect the performance, leading to a complete inhibition of spatial learning These results are in agreement with several earlier reports, even though in these studies cognitive and motor effects were not equally well separated

In these studies mainly the method of lesioning dopamine pathways or brain areas was applied Lesions with 6-hydroxydopamine (injected either into the ventricle or directly into the nigrostriatal bundle [28]) or with ibotenic acid (injected into the neostriatum [29] or into the accumbens [1]) produced severe resp moderate impairments in the acquisition of the watermaze task with an invisible platform But also in the version with a visible one acquisition was blocked (after 6-OHDA) or impaired (after neostriatal ibotenic-acid lesion) Some authors have reported effects of dopaminergic agents on the performance in the Morris maze with an invisible platform So, Taghzouti et al [24] showed an impairment in the acquisition of spatial learning after a long lasting blockade of dopamine receptor activity within the accumbens And likewise, deficits were reported after systemic or local injections with dopaminergic antagonists by Scheel-Kruger et al [20, 21] These latter authors e g mentioned to have found impairments after haloperidol in the range of 0 1 to 0 5 mg/kg The subtle differences at lower doses found in our study can be due to the use of a larger pool

In describing and discussing their results, the above mentioned authors all suggested that not the spatial abilities per se were abolished but rather that the capacity of the animal to use distal cues for guidance or to select alternative strategies was diminished In our experiments, the large effect of haloperidol on every block's first trial, especially at the dose of 0 04 mg/kg, might be due to a diminished ability of the animals to select arbitrarily the best strategy for searching for the invisible platform, as a consequence of an impaired neostriatal functioning This explanation is in accordance with the findings of Cools [9] that both haloperidol at the dose of 0 04 mg/kg and neostriatally applied haloperidol reduced the ability to select the best

strategy in a stressful situation At a dose of 0 07 mg/kg, an impaired capacity to make use of (distal) cues for guidance to the platform throughout all trials might be added, due to a diminished functioning of the nucleus accumbens

In conclusion, haloperidol at low doses specifically impaired the acquisition of spatial learning In future research, it remains to be investigated whether we can establish a differential role for either one or both the striatal areas in this version of Morris water maze learning

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CHAPTER 4.

SPATIAL LOCALIZATION IN THE MORRIS WATER MAZE IN RATS: ACQUISITION IS AFFECTED BY INTRA-ACCUMBENS INJECTIONS OF THE DOPAMINERGIC ANTAGONIST HALOPERIDOL

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ABSTRACT

Previous studies (Ploeger et al , 1992) showed that low doses of systemically injected haloperidol affected spatial learning in the Morris water maze This study investigated effects of intra-accumbens injections of haloperidol on spatial learning To control for motivation and sensorimotor coordination, rats were trained to escape onto a visible platform Low doses (50-100 ng) of haloperidol impaired spatial learning, whereas escaping on a visible platform was undisturbed Haloperidol 500 ng completely blocked acquisition, because of combined learning and motor-impairments Retrieval of an acquired escape response was unaffected by haloperidol 500 ng The data show that mesolimbic dopaminergic activity is involved in the acquisition of spatial localization The results are related to studies demonstrating the involvement of the nucleus accumbens in cue-directed behaviours

INTRODUCTION

Recently we demonstrated that the dopaminergic antagonist haloperidol impairs spatial learning (Ploeger, Spruijt, & Cools, 1992) Haloperidol, applied systemically in low doses, caused deterioration in performance in the Morris water maze with an invisible platform, whereas performance in a water maze with a visible platform was not influenced Higher doses induced motor disturbances, which interfered with performance in the spatial task Because of the systemic injections, it could not be assessed which of two main dopaminergic brain areas, namely the ventral (nucleus accumbens) or the dorsal (nucleus caudatus) striatum, mediated the observed effects

Both striatal areas have been implicated in motor behaviour as well as more cognitive functions (Beninger, 1983, Cools & Jongen-Relo, 1991, Divac & Öberg, 1979, Salamone, 1992, Solomon & Staton, 1982) In a review Oades (1985) proposed that dopamine plays a role in the ability of an animal to switch behaviour Previous studies from our laboratory have established differential effects of dorsal and ventral striatal dopamine on this ability Dorsal striatal dopaminergic activity appears to affect switching of behaviour directed by factors intrinsic to the animal (non cue- directed) (Bercken & Cools, 1982, Vrijmoed-De Vries & Cools, 1986) On the other hand, increased dopaminergic activity in the nucleus accumbens enhances the display of different behavioural items guided by external cues in a (one-trial) swimming test (Bos, 1991, Bos, Charria Ortiz, Bergmans, & Cools, 1991)

Morris (1981, 1984) suggested that spatial learning of rats in the water maze depends on the presence of environmental cues Ploeger, Spruijt & Cools (1992) demonstrated that reducing the availability of extra-maze cues interfered with spatial navigation, causing rats to have more difficulty in finding the platform O'Keefe and Nadel, elaborating a theory on spatial navigation (1978), argued that it represents a specific ability, which involves learning to identify places using a mapping system based on information provided by objects in the environment and their spatial relationship Such a mapping system enables an animal to navigate from different starting points directly to a location relative to environmental cues (allocentric spatial navigation) O'Keefe and Nadel (1978) suggested that the hippocampus is responsible for this so-called *locale system* There is now a large body of evidence supporting the involvement of the hippocampus in both the acquisition and retention of allocentric spatial localization, in the Morris water maze (Morris, 1984, see Brandeis, Brandys, & Yehuda, 1989, Morris, Garrud, Rawlins, & O'Keefe, 1982, Morris, Hagan, & Rawlins, 1986, Sutherland, Kolb, & Whishaw, 1982, Sutherland, & McDonald, 1990, Sutherland, & Rodriguez, 1989) On the other hand, the hippocampus is not involved in escaping onto a platform protruding just above the water (Morris, Garrud, Rawlins, & O'Keefe, 1982) Learning to escape on such a visible platform is straightforward, because the rat can escape by simply heading toward and climbing onto this beacon (an example of the *taxon-strategies* (O'Keefe and Nadel, 1978), also called *proximal cue-learning*, (Morris, 1981))

The nucleus accumbens is known to receive glutamatergic afferents from the hippocampus (subicular and CA1 regions) in a topographic manner, as evidenced by anatomical (Groen &

Wyss, 1990, Groenewegen, Vermeulen-VanderZee, Tekortschot, & Witter, 1987, Kelley & Domesick, 1982, Phillipson & Griffiths, 85, Sesack & Pickel, 1990, Totterdell & Smith, 1989), biochemical (Walaas & Fonnum, 1979) and electrophysiological (DeFrance, Marchand, Sikes, Stanley, & Chronister, 1980, Yang & Mogenson, 1984, 1985 and 1986) studies Also behavioural relations have been found hyperactivity effects induced by different kinds of hippocampal treatment can be modified by intra-accumbens manipulation of dopaminergic or glutamatergic neurotransmission (Emerich & Walsh, 1990, Imperato, Honoré, & Jensen, 1990, Mogenson & Nielsen, 1984) In general, it has been suggested that the accumbens can act as an interface between limbic and motor systems as it receives input from limbic structures such as the hippocampus, and projects to motor-output structures (subpallidal and nigral areas) (Cools, 1988, Cools, Dierx, Coenders, Heeren, Ried, Jenks, & Ellenbroek, 1993, Mogenson, Jones, & Yim, 1980, see also Cools, Bos, Ploeger, & Ellenbroek, 1991)

Based on the above-mentioned evidence from the literature, we hypothesized that the effects of systemically injected haloperidol on spatial learning in the Morris water maze (Ploeger, Spruijt & Cools, 1992) were mainly mediated via the nucleus accumbens It has already been shown that nucleus accumbens lesions impair spatial learning in the Morris maze (Annett, McGregor, & Robbins, 1989, Sutherland, & Rodriguez, 1989) In the present study, we investigated the effects of more subtle manipulation of the dopaminergic activity in the nucleus accumbens, by means of local application of the non-selective antagonist haloperidol, on allocentric spatial navigation Both acquisition and retention were examined and experiments testing the effects on locomotor behaviour (open field, see Beninger, 1983 and 1989) and on motivation and sensorimotor coordination (Morris maze with visible platform, see Morris, 1981 and 1984) were carried out

GENERAL METHOD

Animals and surgery

Male Wistar rats, weighing 200-230 g at the time of surgery, were used in all experiments They were housed in groups of 3 and kept in a temperature- and light-controlled room (reversed light/dark cycle lights on between 20 00 and 08 00 h) Water and food were available ad lib All experiments were carried out between 09 00 and 17 00 h

For implantation of the cannulas the rats were anaesthetized with pentobarbital (Narcovet®, 60 mg/kg i p) and placed in a stereotaxic apparatus Stainless steel cannulas (length 5 mm, diameter 0 5 mm) were bilaterally implanted, aiming at the nucleus accumbens (coordinates A=bregma + 1 6, L=+/- 0 9, H=-0 4 mm, based on the atlas of Konig and Klippel (1963)) The cannulas were inserted under a lateral angle of 10° and fixed onto the skull with stainless steel retaining screws and dental cement (Paladur® and Durelon®) (Bos & Cools, 1989)

One control group was implanted with cannulas, aiming at the dorsal striatum Coordinates for implantation were A=bregma + 1 3, L=+/-2 2 (based on the atlas of Konig and Klippel (1963)), while the length of the cannulas was 4 mm

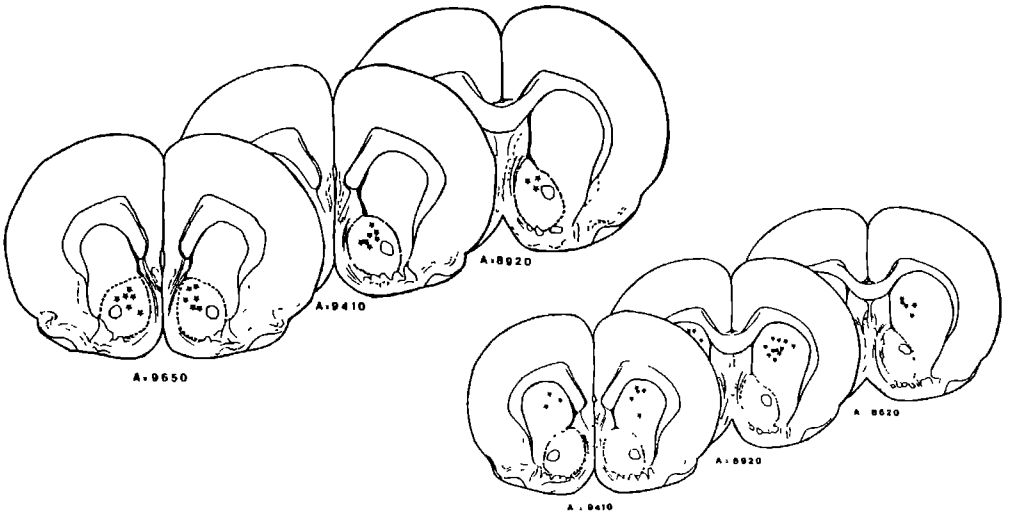


Figure 1. Representative series of injection sites in the nucleus accumbens and the dorsal striatum (lower picture). The planes are taken from König and Klippel, 1963. The A-coordinate is in µm from the interaural line, ranging from 9650 to 8920 for the nucleus accumbens and from 9410 to 8620 for the dorsal striatum.

After surgery, the animals were allowed to recover for at least 10 days. Before starting the experiment the rats were handled on three consecutive days, during which they were habituated to the injection procedure and received a sham injection (on the third day: an injection needle brought in position, no solution injected).

Treatments

Rats were subjected to one of the following treatments: (1) injection with haloperidol in dosages of either 50, 100 or 500 ng per injection-volume (0.5 µl) in the nucleus accumbens; (2) injection with saline into the nucleus accumbens (haloperidol-control); (3) injection with haloperidol 250 ng per injection-volume in the dorsal striatum (control of region) Doses were based on experience from previous experiments at our laboratory. In addition, a non-operated and non-treated group of rats was tested.

For each treatment and for each platform condition, a different group of animals was used and each group consisted of 8-10 rats.

Drug-solution and injection procedure

Haloperidol (stock solution of 5 mg/ml from Janssen Pharmaceutica, The Netherlands) was dissolved in saline.

Injections were given bilaterally, using a Hamilton syringe, with the needle extending 2 mm below the tip of the cannula, thus reaching the nucleus accumbens, or 1.5 mm in the case of the dorsal striatum. One injection-volume of 0.5 µl per side was delivered over a 5 s-period whereafter the needle was kept in place for another 5 s.

Histological verification

After the experiments the animals were sacrificed and the brains removed and fixated with 4% formalin The precise location of the injection sites were determined in serial sections Only the animals with injection sites within the boundaries of the nucleus accumbens (specifically the dorso-medial part), or dorsal striatum (specifically the rostro-dorsal part) were included in the statistical analysis

Figure 1 shows a representative series of injection sites for both the nucleus accumbens and dorsal striatum The legends at the figures 3 and 4 show the exact number of animals per group

Experimental tests and procedures

Open field To test for the effects of haloperidol on locomotor behaviour, the rats were subjected to an open field test The apparatus consisted of a circular box, 75 cm in diameter and surrounded by a 40 cm high dark wall A 12 cm high steel object was placed in the center of the box

The drug (or its solvent) was administered 15 min prior to the test Between injection and testing, the animal remained in its homecage During the test, the rats were allowed to move freely around the open field and to explore the environment and the object for 10 min The path of each animal was automatically recorded and recordings were analysed afterwards (see below)

Morris water maze The apparatus was a black circular pool, 230 cm in diameter and 35 cm deep The pool, filled with water of 26±1 °C to a depth of 23 cm, was located in a large observation room External cues, which were kept constant, surrounded the pool Tests were performed under dim red light conditions One small light near the computerized observation system, necessary for the experimenter, was visible for the swimming animal A radio was on during testing

A small circular escape platform (either a transparent perspex one, located invisibly just below the water surface, or a white one, protruding just above the water, see at Experiment 2) was placed in a constant location in the center of quadrant 1 Four equally spaced points around the wall of the pool were used as starting points

The procedure has been described elsewhere in detail (Ploeger, Spruijt, & Cools, 1992) In short, the rats were given one block of four trials each day, with an intertrial interval of 5-10 min Each trial started from one of four different points, in a semi-random order The drug (or its solvent) was injected every day, always 15 min before the first trial The rat was allowed to swim around until it located the platform, or, when the animal did not find it within 120 s, the rat was placed on the platform by the experimenter The rat was allowed to stay on the platform for 30 s The path of each rat on each trial was automatically recorded and analysed afterwards (see below)

Behavioural recording and statistical analysis

For both the open field test and the Morris water maze task, the path of the animal was automatically registered by a computerized image analysis system Hardware consisted of an IBM AT computer combined with a video digitizer PV VISION PLUS board (Imaging Technology Inc U S A) and a C C D video camera For a detailed description of the software (Noldus Inf Technology B V , Wageningen, The Netherlands), used for data acquisition and analysis, see Spruijt, Hol, & Rousseau, (1992)

In short, a picture of the animal was taken with a sampling method and the coordinates of the position of the rat were determined These coordinates were then stored into the computer (raw data)

Afterwards, the raw data were analysed by computing several parameters, e g latency and travelled distance, for each animal and per group (means and standard errors) Individual values were imported into the statistical package SYSTAT (Wilkinson, Leland, SYSTAT The system for Statistics, Evanston IL SYSTAT, Inc , 1990)

The results of the open field test were statistically analysed by means of an analysis of variance (ANOVA) on one factor For the effect of haloperidol on water maze performance an analysis of variance on one factor for repeated measures was applied When the overall test showed significancy, it was followed by post hoc analysis, Tukey's honestly significant difference (HSD), for assessing differences between specific groups (Pagano, 1986)

EXPERIMENT 1: OPEN FIELD - LOCOMOTION

Method

Four groups of rats were tested one non-cannulated and non-treated control group and three groups with injections into the nucleus accumbens, receiving either saline, haloperidol 100 ng or haloperidol 500 ng

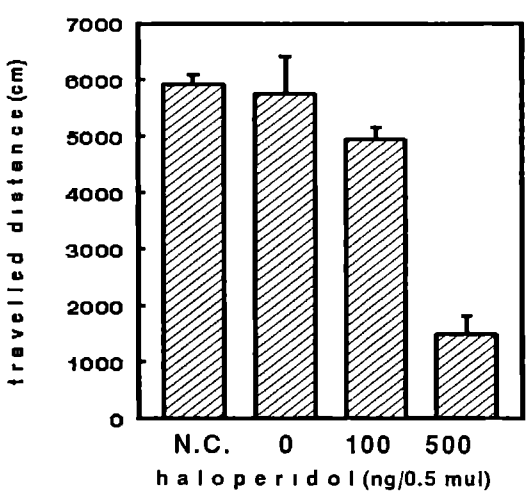


Figure 2. Mean travelled distance (in cm) ± s e m in an open field test Four groups were tested non-cannulated (N C , n=5), saline injected (n=5) haloperidol 100 ng (n=5) and haloperidol 500 ng (n=7)

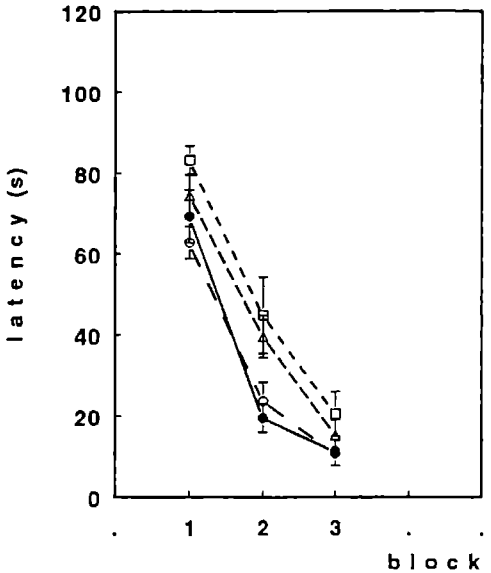


Figure 3. Mean latencies (in s) ± s e m per group and per block of 4 trials, in the Morris maze with a visible platform Control (●, n=9) vs haloperidol 50, 100 and 500 ng (O , n= 7,Δ , n=10,□ , n=8)

Results

Open field data Figure 2 represents the total travelled distance during the 10 min in the open field for each group A significant effect of groups was found, ANOVA F(3,18)= 34 67, p<< 0 01 A post hoc Tukey test showed that haloperidol 500 ng significantly differed from the other three groups (p<0 01)

EXPERIMENT 2: MORRIS MAZE - ACQUISITION

The effects of intra-accumbens dopaminergic manipulation with the antagonist haloperidol on the acquisition of spatial learning were investigated The test was also carried out with a visible platform, examining motivation and sensorimotor coordination

Method

Apparatus and procedure Both a white and visibly located platform and an invisibly located platform from transparent perspex were used Rats were trained for 3 days in the case of the visible task or for 4 days in the case of the invisible task In both platform conditions three doses of haloperidol were tested 50, 100 and 500 ng One non-operated and non-treated control group and one group with cannulas in the dorsal striatum, receiving an injection of haloperidol 250 ng, were trained on the invisible platform condition Training procedure further followed the above given description in the general method

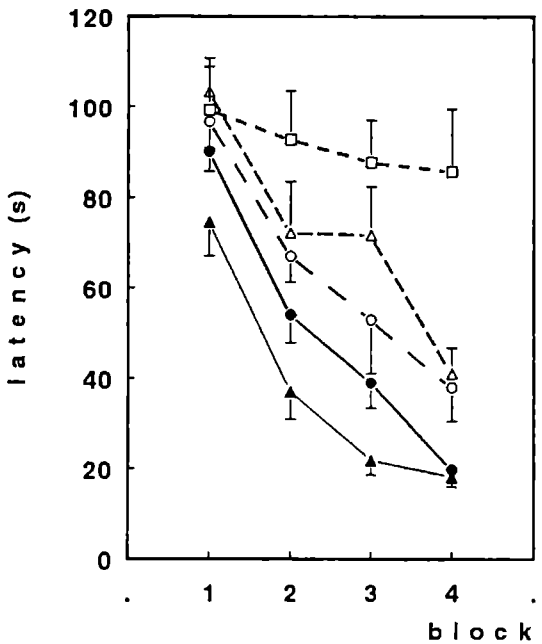
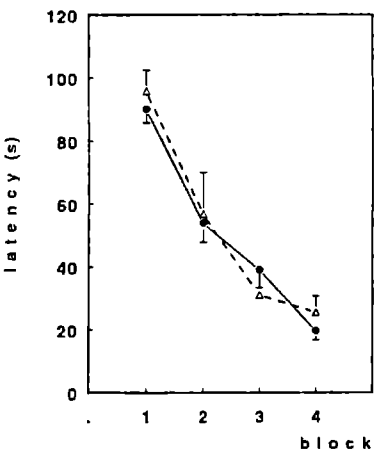


Figure 4. The upper part shows the mean latencies (in s) ± s e m. per group and per block of 4 trials over 4 days, in the Morris maze with an invisible platform, for the nucleus accumbens 5 Groups were tested control without cannulas and no treatment (▲, n=13), cannulated control with saline (●, n=13), and haloperidol 50, 100 and 500 ng (O, n 10, Δ, n=9, □, n=7) An ANOVA yielded a highly significant overall groupseffect, p<<0 01, see text In the lower part latency per block is given for the group with injections in the dorsal striatum (haloperidol 250 ng,Δ, n=9) in comparison to the group with saline-injections in the nucleus accumbens (●, n=13)



Results

Morris maze data Figures 3 and 4 show the performance of differently treated groups of rats in the Morris water maze with a visible platform or an invisible platform. In both figures latencies to find the platform during acquisition are depicted.

With the platform visibly present, all groups showed improvement over days, $F(2,58)= 223.0$, $p<<0\ 01$. With respect to treatment, an overall effect of groups was apparent: $F(3,29)= 3.73$, $p=0.022$. Post hoc analysis showed that haloperidol 500 ng significantly differed from saline (Tukey HSD: $p=0.028$) only in the second block. In the same block a tendency to differ from saline was found for haloperidol 100 ng (Tukey HSD: $p=0.080$).

In the task with the invisibly located platform, all groups showed decreasing latencies with time (ANOVA: $F(3,141)= 58.74$, $p<<0.01$), except for the group treated with the highest dose of haloperidol. This latter group hardly showed any improvement over days (figure 4.a). Highly significant differences in the improvement of performance were found between groups ($F(4,47)= 17.32$, $p<<0.01$) and a significant interaction between group and time ($F(12,141)=$

2 14, p=0 018), mainly due to the absence of improvement in performance in the group treated with 500 ng haloperidol An effect of implantation and injection was present control animals without any treatment showed significantly lower latencies up to block 3 compared with control animals with implanted cannulas and subject to saline injection ($F(1,24)= 10\ 36$, $p<0\ 01$) Both groups treated with 50 ng or 100 ng haloperidol significantly differed from the saline-treated group ($F(1,21)= 4\ 60$, $p=0\ 044$ for haloperidol 50 ng and $F(1,20)= 13\ 39$, $p<0\ 01$ for haloperidol 100 ng)

The effects of haloperidol on performance in the maze with the visible platform significantly differed from those on performance in the maze with the invisible platform ($F(9,195)= 2\ 71$, $p<0\ 01$, interaction of haloperidol-visible platform and haloperidol-invisible platform)

Figure 4 b compares the effect of 250 ng haloperidol injected in the dorsal striatum with a saline injection in the nucleus accumbens The improvement in performance over time was very similar for both groups of rats

EXPERIMENT 3: MORRIS MAZE - WITHDRAWAL AND RETENTION

In this experiment, we studied the effect of withdrawal of haloperidol and the effect of haloperidol on retrieval of an acquired escape response

Method

Subjects and procedure The group trained on the invisible platform-condition and treated with haloperidol 500 ng during 4 days (from experiment 2, n=7) received 2 extra blocks of training (day 5 and 6) First, no haloperidol was given on day 5 On day 6 (after the animals had acquired the escape response) application of haloperidol 500 ng was reinstated

Results

Figure 5 shows the effect of withholding haloperidol in the fifth block after 4 days of injection with 500 ng haloperidol Once haloperidol was no longer injected, latencies decreased significantly during the trials of day 5 ($F(1,12)= 5\ 40$, $p=0\ 038$) The low latencies in trial 19 and 20 (fig 5 b) indicate that the animals learned to locate the hidden platform

Subsequently, a new injection of 500 ng haloperidol prior to block 6 did not alter the acquired escape response latencies on day 6 did not significantly differ from latencies during trial 19 and 20 on day 5 (see fig 5 b)

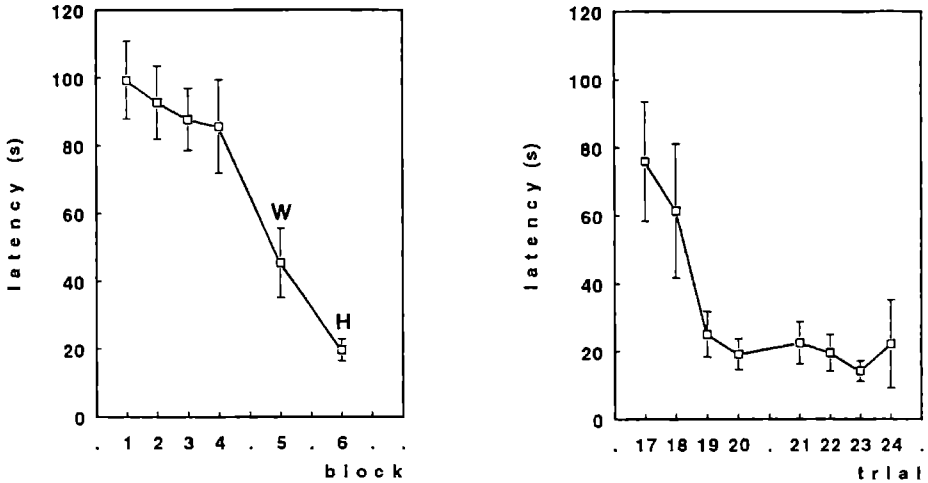


Figure 5. Effect of withdrawal (W) from drug-treatment and the effect of haloperidol on retrieval of information (H). Animals (n=7) were treated with haloperidol 500 ng during block 1-4. No drug-injection was given prior to block 5 (trial 17-20, right panel), while an injection of haloperidol 500 ng was again applied prior to block 6 (trial 21-24, right panel).

GENERAL DISCUSSION

Our objective was to establish by which of the main dopaminergic areas the effects of systemically applied haloperidol on Morris maze behaviour (Ploeger, Spruijt, & Cools, 1992) are mediated. In this study we investigated a possible role for the nucleus accumbens in spatial learning.

The open field experiment showed that haloperidol 100 ng did not significantly decrease locomotor activity, in contrast to the clear motor effect of haloperidol 500 ng. So, we considered the range of doses from 0 to 500 ng of haloperidol appropriate in order to dissociate possible cognitive effects from motivational and sensorimotor effects of haloperidol in the Morris maze.

Cannulation and injection procedure influenced the performance of the rats in the Morris maze with an invisible platform. Cannula and injection needle, running through cortical and striatal areas, cause some damage. Without additional control experiments, we can only speculate about the precise relation between the location and extent of the damage and the effect on spatial learning. The damage caused in the striatum may have been largely responsible for the observed 'procedural' effects. However, the procedural effects were significantly smaller than those observed after injections with haloperidol.

Haloperidol dose-dependently impaired acquisition of spatial localization of the hidden platform. The highest dose (500 ng) induced a blockade of acquisition, whereas disturbances of the swimming behaviour appeared in the course of training (reduction of swimming speed, floating and treading the water). However, at the same doses, the animals were at most only mildly and transiently impaired in the task with the visible platform. The similarity of response requirements and motivation in the two Morris maze tasks and the mild and transient effects in the visible platform task imply that the effects on spatial learning after haloperidol (even after 500 ng) were primarily due to an effect on spatial learning.

Well-trained animals could no longer be influenced by dopaminergic manipulation (see experiment 3) This is in agreement with results obtained earlier with systemic injection of haloperidol (unpublished personal observations) It is also in line with a study from by Sutherland and Rodriguez (1989), reporting that electrolytic lesions have no deteriorating effect on performance in animals trained prior to surgery Thus, intra-accumbens dopaminergic activity does not play a role in the process of retention and/or retrieval of information This observation further supports that the effect of a high dose of haloperidol on the acquisition of spatial learning is mainly related to spatial learning and not to other possible effects

A dose of 250 ng of haloperidol injected in the dorsal striatum (into the dopaminergic region, see Vrijmoed-de Vries & Cools, 1986) did not impair localization of the invisible platform, indicating that the effects of intra-accumbens injections of haloperidol were indeed mediated by this nucleus In the previous study (Ploeger, Spruijt, & Cools, 1992), we speculated about a combined influence of both the nucleus accumbens and dorsal striatum The similarities between the effects of systemic and intra-accumbens injections of haloperidol point to an important role for the accumbens in mediating the effects of haloperidol

This does not yet exclude the possibility that the dorsal striatum plays a role in spatial learning The effects of a wider range of doses of haloperidol, injected in the dorsal striatum, were examined in a follow-up study and discussed separately (Ploeger et al , 1994)

The present results are in agreement with those from lesion experiments Bilateral electrolytic lesions in the accumbens impaired place learning over many trials (Sutherland, & Rodriguez, 1989) In contrast, lesioned animals were capable of escaping onto a visible platform Post-training lesioning affected performance only on the first trial The deficit in acquisition was rather similar to that of animals with fimbria/fornix lesions Sutherland and Rodriguez concluded that place learning required both an intact hippocampal circuit as well as a normally functioning accumbens Also animals with ibotenic acid lesions in the nucleus accumbens showed impaired spatial learning, although in this case the impairment was less severe and less persistent (Annett, McGregor, & Robbins, 1989) The effects of haloperidol in our study are more similar to the effects of the ibotenic acid lesions Electrolytic lesioning of the accumbens led to a more pronounced deterioration, possibly because electrolytic lesions have a stronger damaging effect (Sutherland, & Rodriguez, 1989) Furthermore, the animals in our study were monitored for only four blocks (in which period non-treated controls can reach asymptotic performance), whereas the animals in the above cited lesion studies were trained for ten blocks Therefore, we cannot tell whether or not our haloperidol-treated animals eventually would have reached control level, as the animals in the study of Annett et al did

The present results indicate that in addition to the involvement of the nucleus accumbens in spatial learning, more specifically the dopaminergic activity in the accumbens is involved Previous studies at our laboratory demonstrated effectiveness of both the non-selective antagonist haloperidol and the D2-antagonist raclopride in the display of cue-directed behaviours in a one-trial swimming test (Bos, 1991) Further studies are required to

investigate the roles of specific dopaminergic receptor-systems in the ventral striatum in spatial learning By means of systemic application D1 (SCH 23390) and D2 (raclopride) antagonists have already been demonstrated to be effective in the Morris water maze (Scheel-Kruger, Widy-Tyszkiewicz, & Krieger, 1990) Additional experiments are also needed to relate the effects of intra-accumbens dopaminergic manipulation on spatial learning more strictly to its role in the display of cue-directed behaviours Experiments in which specific cues control spatial localization as well as in which localization would be based on fixed routes (no cues present in the environment) could be conducted In the latter case it is expected that intra-accumbens manipulation of the dopaminergic activity has no effect on performance

In conclusion, low doses of the dopaminergic antagonist haloperidol applied into the nucleus accumbens specifically impaired the acquisition and not retention of spatial navigation It is suggested that the involvement of ventral striatal dopaminergic activity in spatial learning is associated with its involvement in displaying behaviour directed by external cues

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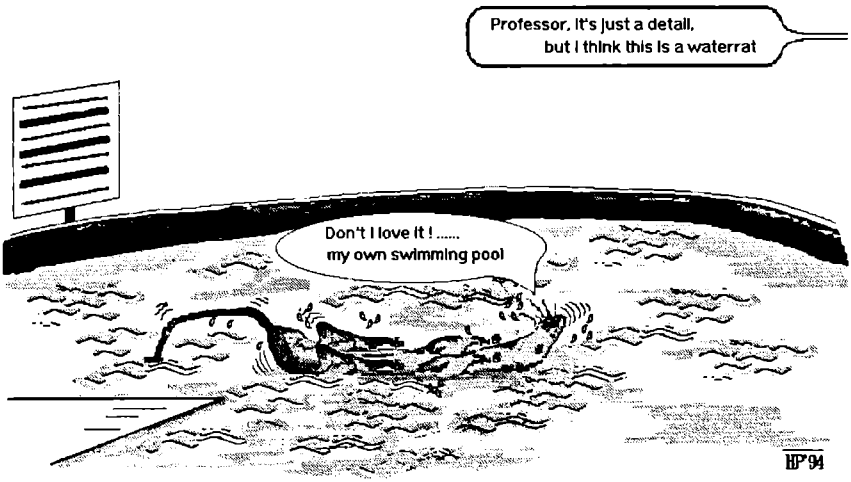
Professor, it's just a detail,
but I think this is a waterrat
Don't I love it ! .....
my own swimming pool
HP'94

CHAPTER 5.

STRIATALLY ADMINISTERED HALOPERIDOL DOES NOT SPECIFICALLY AFFECT ALLO- OR EGOCENTRIC SPATIAL LOCALIZATION IN WATER MAZES

G.E. Ploeger, B.M. Spruijt, N M. van Duursen and A.R. Cools

Submitted

ABSTRACT

Systemic or intra-accumbens administration of the dopaminergic antagonist haloperidol has been found to impair spatial learning in the Morris maze task[18 19] The present study investigates the extent to which administration of haloperidol into the dorsal striatum affects allocentric and egocentric spatial behaviour in the Morris maze task and a water T-maze respectively A disrupting effect of cannulation and the injection-procedure was found A low dose of 250 ng did not further disturb localization of a hidden platform as compared to operated controls, while higher doses (≥ 375 ng) impaired this, but not in a dose-dependent manner Furthermore, also escaping onto a clearly visible platform was deteriorated at these latter doses, indicating sensorimotor disturbances Egocentric localization in a simple water T-maze was unaffected at doses of 250-325 ng In conclusion, this study shows that administration of the dopaminergic antagonist haloperidol in the chosen doses in the dorsal striatum does not specifically affect allo- and egocentric localization in spatial water-tasks where impairments were produced, they were dose-independent and co-occured with sensorimotor deficiencies

INTRODUCTION

Animals can use different strategies for localizing important places *Allocentric* spatial localization is thought to involve the construction of a map of the environment, containing information about places in relation to externally available objects or cues ('locale strategy', see [14]) Performance in the classic Morris water maze constitutes a clear example of allocentric spatial behaviour in finding a hidden platform animals rely on the presence of environmental (distal) cues[14 16]

Egocentric spatial localization makes use of a sequence of changes in the orientation of the body axis to lead the animal to its goal (one form of a 'taxon strategy'[14], also called position-response) In a radial or T-maze an animal can be forced to acquire a position-response to find food on a location that is always within a fixed distance and direction in relation to the animal's own body (at the start of the test), it will have to execute a specific set of responses Such a position-response is not controlled by external cues, but is assumed to be based on factors intrinsic to the animal

We have demonstrated that systemically applied haloperidol induces a learning deficit in the Morris water maze task[18] Low doses of this dopaminergic antagonist attenuate performance in the spatial version (invisible platform), while leaving performance in the non-spatial form of this task (visible platform) intact With higher doses motor disturbances appear, interfering with the learning impairment and leading to further deterioration

In order to determine the respective contribution of the two main dopaminergic brain areas (ventral versus dorsal striatum), effects of manipulation of these two areas are examined separately The present study deals with the role of the dorsal striatum

The ventral striatum, especially the nucleus accumbens, has been shown to play a role in the acquisition of allocentric spatial localization in the Morris maze both lesions of the accumbens[1 25] and intra-accumbens injections with the dopaminergic antagonist haloperidol[19] impair allocentric spatial learning in the Morris maze This involvement of the nucleus accumbens becomes understandable in the light of its postulated role in the display of cue-directed behavioural items[2 4 5] (see also [10 11]) For example, enhancement of the dopaminergic activity within the nucleus accumbens leads to an enhancement of the number of different cue-directed behaviours in a one-trial, forced-swimming task[5]

In contrast, the dorsal striatal dopaminergic activity has been implicated in switching to behaviour controlled by factors intrinsic to the animal (arbitrarily)[7 26 27] An injection of a dopamine agonist into the dorsal striatum enhances the animal's ability to select arbitrarily (that is, not directed by external stimuli) the best (life-saving) strategy to cope with the stressfull situation of the above mentioned forced-swimming test[7] In view of this opposite function, no active role in allocentric spatial navigation in the Morris water maze is expected for the dorsal striatum This inference is examined in the first part of this study

On the other hand, the dorsal striatum might be expected to contribute to the acquisition of position-responses First, a position-response requires to be carried out without the help of externally present cues and is assumed to be based on intrinsic factors (see [21]) Second,

previous reports from the literature indicate that lesions of the dorsal striatum impair the acquisition of egocentric position responses[6 15 20 21] Moreover, adding salient visible intra-maze or distal, external stimuli to the egocentric response task, diminishes this impairment[15] So, examination of the hypothesized involvement of dopamine in the dorsal striatum in the acquisition of egocentric spatial localization is carried out in the second part of this study This latter is done in a water T-maze to motivate the animals in both paradigms in a similar way As in our previous study on the nucleus accumbens, we manipulate the dopaminergic activity by employing local injections of the dopaminergic antagonist haloperidol

A Morris water maze with a visible platform is used as a control-task Such a task constitutes another example of taxon-strategies (guidance-strategy, see [14]) the visible platform acts as a clearly visible stimulus and the goal-object at the same time and the animal is able to approach this obvious object by any particular behaviour This task is applied here to check whether the animal has sufficient sensorimotor capacities at its disposal[17]

MATERIALS AND METHODS

Animals and surgery

Male Wistar rats, housed in groups of 3 and kept in a temperature and light-controlled room at a reversed light/dark cycle (lights on between 20 00 and 08 00 h), were used All experiments were carried out between 09 00 and 17 00 h Water and food were available ad lib Per treatment, a group of 8-10 animals was used

For implantation of the cannulas, the rats (weighing 200-230 g) were anaesthetized with pentobarbital (Narcovet®, 60 mg/kg i p) In the case of the T-maze experiments, a neurolept-analgeticum (Hypnorm® 0 8 ml/kg i m + Stesolid®, 0 1 ml/kg) was applied, for practical reasons The rat was then placed in a stereotaxic apparatus Stainless steel cannulas (length 4 mm, diameter 0 5 mm) were bilaterally implanted, aiming at the dorsal striatum (coordinates A=bregma + 1 3, L= +/-2 1, based on the atlas of Konig and Klippel[13]) The cannulas were fixed onto the skull with stainless steel retaining screws and dental cement (Paladur® and Durelon®)[3]

After surgery, the animals were allowed to recover for a period of at least 10 days Before starting the experiment the rats were handled on three consecutive days, during which they were habituated to the injection procedure and received a sham injection (an injection needle brought in position, no solution injected)

Injection procedure, drug-solution and histological verification

Injections were given bilaterally, using a Hamilton syringe, with the needle extending 1 4 mm below the tip of the cannula, thus reaching the dorsal striatum A volume of 0 5 µl per side was delivered over a 5 s-period whereafter the needle was kept in place for another 5 s Injection was given each day, 15 min before the first trial (see below)

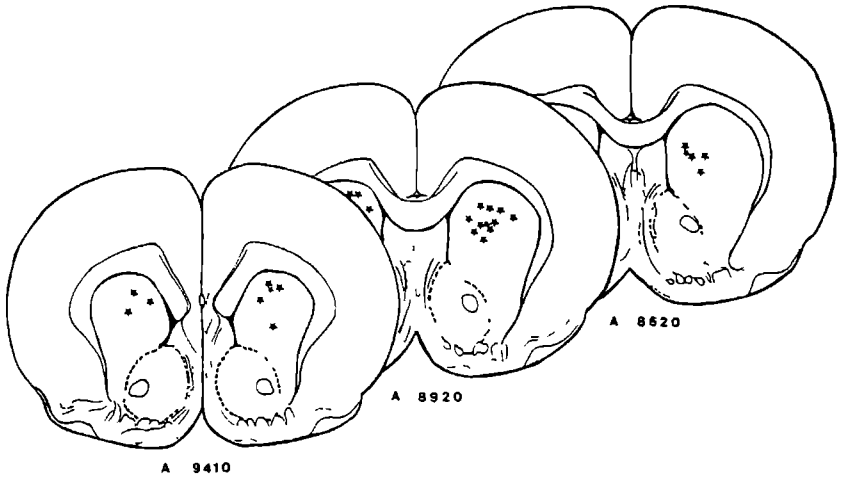

Figure 1 Representative series of injection sites in the neostriatum The planes are taken from König and Klippel [1] The A-coordinate is in µm from the interaural line

The dopamine D2 antagonist haloperidol (using a stock solution (5 mg/ml) from Janssen Pharmaceutica, The Netherlands) was dissolved in saline The doses ranged from 250 to 500 ng (see below) per injection-volume of 0 5 µl per side A saline injection served as control

After the experiments, the animals were sacrificed and the brains removed and fixated with 4% formalin The precise location of the cannulas and injection sites were determined in serial sections Only the animals with injection sites within the rostral part of the dorsal striatum were included in the statistical analysis

Apparatus and experimental procedures

Experiment 1: Morris water maze

The apparatus was a black circular pool, 230 cm in diameter and 35 cm deep The pool, filled with water of 26 ± 1 °C to a depth of 23 cm, was located in a large observation room

External cues, that were kept constant, surrounded the pool Behavioural testing was performed under dim red light conditions, with one small light on near the computerized observation system for use of the experimenter

Both a visible white platform, protruding just above the water surface (non-spatial task), and an invisible transparent perspex one, hidden below the surface (for the 'distal cue' or spatial task), were used The platform, whether visible or invisible, was placed in a constant location in the center of quadrant 1 Four equally spaced points around the wall of the pool were used as starting points

The procedure has been described elsewhere in detail[18] In short, animals in groups of four were given a block of four trials each day, with an intertrial interval of 5-10 min Each trial started from one of four different points, in a semi-random order The drug (or its solvent) was injected every day, always 15 min before the first trial The animal was allowed to swim around until it located the platform, or, when the rat did not find it within 120 s, it was placed on the

platform and left there for 30 s The animals were trained for 3 days on the visible task, while for the invisible task, they were given 4 days of training Apart from saline, haloperidol 250 ng (spatial task only), 375 and 500 ng was examined Performance was compared to that of a non-operated, non-treated group

Furthermore, the effect of haloperidol injections into the dorsal striatum on performance of well-trained animals was studied Therefore, 8 animals that were used as controls during acquisition (block 1-4) and had received saline-injections, were used for three more blocks These blocks were given 2 weeks after the acquisition-training and consisted of 3 trials each First they were again trained on the task with the invisible platform on block 5 and 6, in order to obtain stabilized performance Then, on the next day, the animals received an injection of haloperidol 500 ng into the dorsal striatum 15 min prior to testing on the 7th block

Experiment 2 water T-maze

A grey plastic T-maze was positioned within the above-described circular pool The T-maze was 40 cm high, thus extending 15 cm above the waterlevel, 20 cm wide and consisted of one, 90 cm long start-arm and two, 80 cm long goal-arms A black piece of plastic was put on top of the walls (but leaving open all three arm-endings) Furthermore, the T-maze was rotated throughout the experiments, so that there was no constant relation between the position of the maze and external stimuli Behavioural testing was performed under dim red light conditions, and recording of the paramaters was done by hand

An invisible platform was placed at the end of one of the goal-arms, for the animal to escape onto In order to find this platform the animal had to turn always either left or right, when coming out of the start-arm Thus, the animal had to learn the location of this place of escape in relation to its own body-axis (egocentric)

The animals were given 1 block of 5 trials each day The drug (or its solvent) was injected every day, always 15 min before the first trial The animal was gently put into the water, at the beginning of the start-arm and facing the wall of the pool On the first day, trial 1 was used to determine the preference side of each rat In the next trials the platform was always put at the end of the opposite goal-arm In the second trial (day 1) the animal was forced to 'choose' the correct arm, because the other (preference arm) was closed on forehand From then on, a non-correction, choice procedure was applied The animal was allowed to swim through the arm and to choose to enter one of the goal-arms When the animal chose the correct one (always the same direction for the same animal) and escaped onto the platform, it was immediately taken out When it swam into the incorrect arm, a door was put into the water (by hand) behind the animal to close the alley The rat was forced to swim around for 30 s in this arm without platform, before it was relieved from the water Animals were tested in groups of four, when all four rats had received trial 1, the T-maze was rotated and trial 2 was given, and so on The rats were trained until they had reached a criterion of at least 4 correct choices out of 5 trials ($\geq$ 80%) on 2 consecutive days

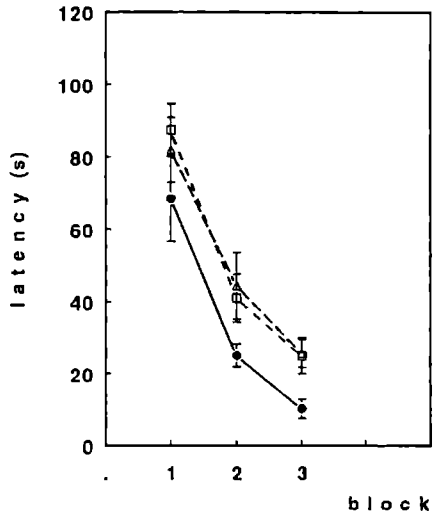

Figure 2. Mean latencies (in s) ± s e m per group and per block of 4 trials, in the Morris maze with a visible platform Three groups are depicted control (●), haloperidol 375 (Δ) and haloperidol 500 ng (□), n= 8 per group

Both saline and haloperidol, 250 and 325 ng, were tested These doses were based on the results in the Morris maze with visible and invisible platform (see below, at the results) Furthermore, a non-operated and non-treated group was used

Behavioural recording and analysis

In the case of computerized recording (only used in the Morris maze experiments), the path of the animal was automatically registered by an image analysis system Hardware consisted of an IBM AT computer combined with a video digitizer PV VISION PLUS board (Imaging Technology Inc U S A) and a C C D video camera See Spruijt et al [23] for a detailed description of the software (Noldus Inf Technology B V , Wageningen, The Netherlands), used for data acquisition and analysis

In short, a picture of the animal was taken with a sampling method and the coordinates of the position of the rat were determined These coordinates were then stored into the computer (raw data)

The raw data were analysed afterwards and various computations were made So, latency was calculated for each animal and per group (means and standard errors) Individual values were then imported into the statistical package SYSTAT (Wilkinson, Leland, SYSTAT The system for Statistics, Evanston IL SYSTAT, Inc , 1990)

For analysing the effect of haloperidol on water maze performance, an analysis of variance on one factor for repeated measures was applied When the overall test showed significance, it was followed by post hoc analysis (Tukey HSD) for assessing differences between specific groups

In the T-maze experiments, scoring was done by hand Latency to escape onto the platform was measured and errors (entering the incorrect arm) were counted The percentage of correct responses as well as the number of blocks needed to reach criterion were calculated Statistically, the results were analysed by means of an ANOVA, where appropriate, the option of repeated measures was applied or post hoc analysis was performed

RESULTS

Histology

A representative series of injection sites is shown in figure 1 Only animals with injections that were confined to the rostral part of the dorsal striatum, were included for further analysis See the legends from the figures for the exact number of animals per experimental group

Experiment 1. Morris water maze

Figures 2 and 3 show the performance of differently treated groups of animals in the Morris water maze with the visible platform (fig 2) and with the invisible one (spatial version, fig 3) In both figures latencies to find the platform during acquisition are depicted

In the case of the Morris maze with the visible platform, saline and two doses of haloperidol (375 and 500 ng) were tested All groups showed improvement over days, $F(2,42)= 89\ 263$, $p<<0\ 01$ An overall ANOVA with repeated measurements yielded a near significant effect of groups $F(2,21)=3\ 349$, $p=0\ 055$ Both groups differed significantly from the control $F(1,14)=4\ 673$, $p=0\ 048$ for haloperidol 375 ng and $F(1,14)=6\ 517$, $p=0\ 023$ for haloperidol 500 ng, but the graphs of the haloperidol-groups did not reveal a dose-dependent relationship

For the task with the invisible platform (spatial learning), five groups were compared two controls either without cannulas and non-treated or with cannulas and saline-injected, and three haloperidol-groups treated with increasing doses of 250, 375 and 500 ng/side For all groups, latencies clearly decreased with time (ANOVA $F(3\ 144)=76\ 636$, $p<<\ 0\ 01$) Furthermore, a highly significant group-effect was demonstrated by an overall ANOVA, $F(4,48)=20\ 082$, $p<<\ 0\ 01$, no interaction-term was found

Examining the performance of the different groups in more detail, an effect of the cannulas and injection procedure appeared the cannulated and saline-treated group differed significantly from the non-treated one ($F(1,24)=15\ 807$, $p<0\ 01$) It is noted here that in the

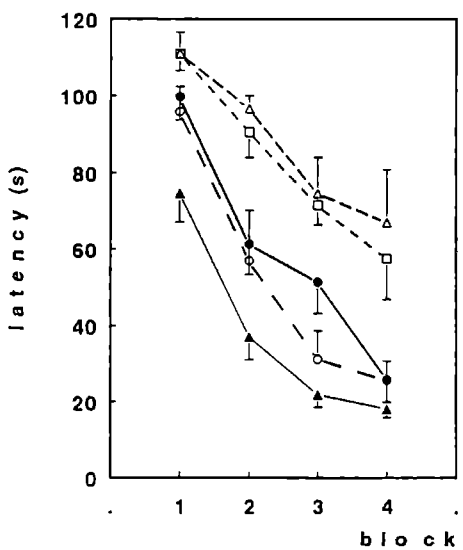

Figure 3 Mean latencies (in s) ± s e m per group and per block of 4 trials over 4 days, in the Morris maze with an invisible platform Five groups were tested control without cannulas and no treatment (n=13, ▲), cannulated control with saline (n=13, ●), and haloperidol 250, 375 and 500 ng (n= 9, O, n=9, Δ, n=10, □) An ANOVA yielded a clear groupseffect, see text

case of the visible platform no effect of surgery and injection was seen (data not shown) Furthermore, analysis showed that whereas haloperidol 250 ng did not impair maze performance as compared to the saline-group ($F(1,19)=0\ 365$, $p=0\ 553$), haloperidol 375 and 500 ng enhanced latencies to escape onto the platform ($F(1,20)=12\ 428$, $p<0\ 01$ respectively $F(1,21)=12\ 656$, $p<0\ 01$), but also in this case there was no dose-dependent relationship

The effect of haloperidol on retention in well-trained animals is presented in figure 4 After the animals had achieved good performance (block 6), haloperidol 500 ng did not affect escaping onto the platform as the mean latency of block 7 (trial 23-25) shows

Experiment 2: water T-maze

In the egocentric localization task within the water T-maze, four groups were examined a non-operated control, a cannulated and saline-injected group and two haloperidol-groups (250 and 325 ng) We chose to apply these doses of haloperidol, because higher doses of haloperidol impaired escaping onto the visible platform in the Morris maze, indicating the presence of sensorimotor disturbances (see discussion)

As it was less difficult to find the platform in the T-maze, latencies to escape were significantly lower than in the Morris water maze, from the first trial on They decreased from around 20 s in block 1 to about 10 s in the last block

Two other parameters are represented in figure 5 and 6

First, the mean percentage correct responses per block for all four groups is depicted in fig 5 An overall ANOVA showed a significant effect of groups ($F(3,229)=3\ 081$, $p=0\ 043$), but post hoc analysis demonstrated a difference only between the non-operated group and haloperidol 325 ng on block 2 (Tukey test $p=0\ 047$) Significancy was further found between the non-

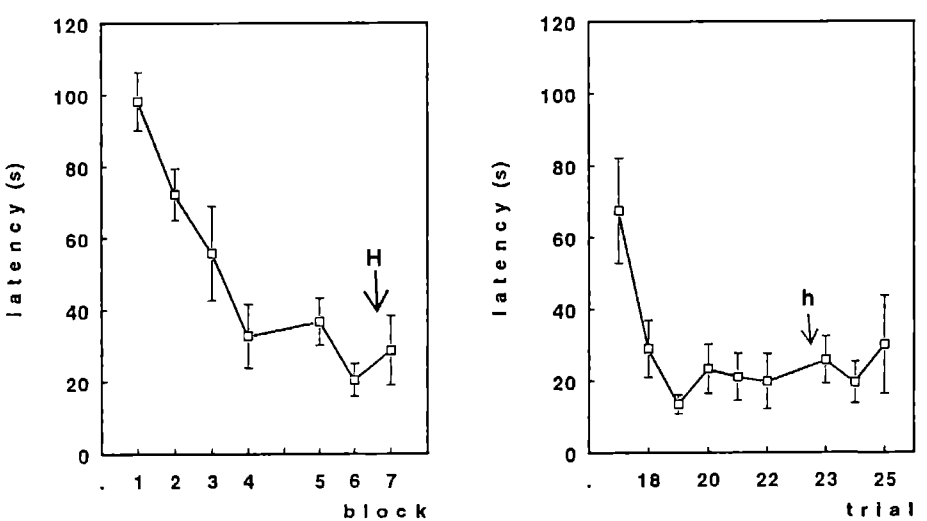

Figure 4 Effect of haloperidol 500 ng, injected into the neostriatum, in pretrained animals (for training-schedule see text) In the left graph latencies per block are depicted, while in the right part the results for block 5, 6 and 7 are shown in more detail (latencies per trial (17-25)) Number of animals in this group was 8 Haloperidol is given just before the 7th block (H), that is before trial 23 (h, see arrows)

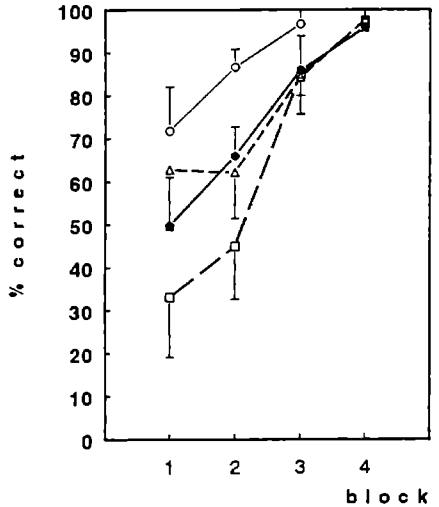

Figure 5. Mean percentage correct responses (± s e m) per group per block in the T-maze A non-treated control-group (n=6 O), a saline-injected one (n=10, ●) and two haloperidol groups 250 ng (n=9, Δ) and 325 ng (n=8, □) were tested

operated animals and the saline-treated rats in a separate ANOVA F(1,14)=6 726, p=0 021
The mean total number of errors made until the criterion was reached is demonstrated in fig 6 An effect of groups was found ANOVA F(3,29)=2 867, p=0 054 A post hoc Tukey test yielded a difference between the non-operated animals and haloperidol 325 ng (Tukey test p=0 032) Furthermore, a separate ANOVA stressed the difference between the non-operated and the saline-treated animals F(1,14)=6 724, p=0 021
However, all groups required the same number of blocks (days) to attain good performance (see block 4 in fig 5) In this experiment too, the effects of haloperidol 250 ng did not differ from those of the cannulated and saline-injected group

Discussion

With regard to the performance in the Morris water maze with the visible platform (non-spatial), both haloperidol 375 and 500 ng were effective latencies to find and climb onto the platform were enhanced as compared to those of control (saline-injected) animals However, both doses of haloperidol impaired performance in this task to the same extent (no dose-dependent relationship) Furthermore, starting with enhanced latencies at the first block, the haloperidol-groups improved over time (i e learned) in a similar way as the saline-group Saline-injected animals performed similar to non-treated animals
Haloperidol 375 and 500 ng also affected latencies to escape onto a hidden platform (spatial learning) In this case too, the enhancement of the latencies was comparable for both doses On the other hand, spatial learning in the group tested with the lower dose of 250 ng did not differ from that shown by the saline-injected animals These operated and saline-injected animals, however, did differ in performance from non-operated and non-treated rats A similar cannulation/injection-procedure effect has been found earlier[7]
Taken together, this means that damage of the dorsal striatum as a consequence of just the cannulation/injection-procedure impairs performance in the spatial Morris maze task Haloperidol 250 ng does not give further impairment Stronger inhibition of the striatal

dopaminergic activity by means of higher doses of the antagonist leads to disturbances in both tasks to a similar extent and in a non dose-dependent way This latter finding indicates that effects of dopaminergic manipulation on spatial localization per se cannot be distinguished from effects on sensorimotor abilities[17]

In our study on the nucleus accumbens[19], these effects could be separated low doses of haloperidol specifically affected spatial learning, leaving non-spatial learning intact, whereas a higher dose, in addition, elicited sensorimotor deficiencies Two additional differences between the results of the studies on the nucleus accumbens and the dorsal striatum are noteworthy First, while in the nucleus accumbens doses of about 100 ng of haloperidol were effective in attenuating spatial learning, only doses higher than 250 ng were effective in the dorsal striatum Second, the haloperidol effects were dose-dependent in the case of the nucleus accumbens, whereas they were dose-independent in the present study Together, these differences point out that mesolimbic dopaminergic activity is more specifically involved in allocentric (cue-dependent) spatial learning than the dorsal striatum, as was expected from the functional difference between these brain areas (see Introduction)

Nevertheless, manipulations of the dorsal striatum did have effects in the acquisition-phase of non-spatial and spatial navigation (in contrast to the retrieval-phase, see below), these need to be considered more closely Both the effects of haloperidol 375 and 500 ng and of the damage due to cannulation and injection (only present in the spatial learning task) indicate that, during the acquisition, the dorsal striatum plays somehow a role in the displayed behaviour

According to our hypothesis (see the introduction and [7 26 27]), the dorsal striatum is presumed to allow an animal to switch arbitrarily (that is, not directed by external factors) its behaviour Thus, the dorsal striatum might be active in order to facilitate the arbitrarily selection of appropriate behaviour to cope with the stressfull situation in the Morris maze (as was demonstrated before, in a one-trial forced-swimming test[7]) This will be so, at least, as long as

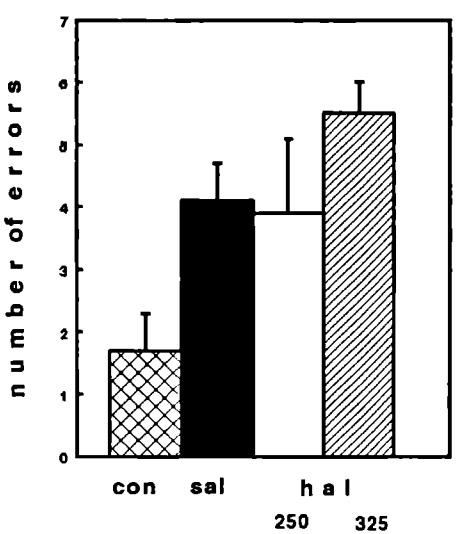

Figure 6 Mean total number of errors per group, made until criterion was reached (see text), in the T-maze Numbers of animals per group can be found in the legend of figure 5

external cues will not yet have gained control over the animal's behaviour in the Morris water maze It is suggested that the cannulation/injection damage affects this function of the dorsal striatum, leading to a less efficient performance in the spatial learning task, while leaving sensorimotor capacities still intact Earlier, Whishaw et al [28] have also suggested that the dorsal striatum is more likely to be involved in the selection between alternative strategies than in navigating per se More precise analysis of swimming patterns should be carried out in order to strenghten this idea

Haloperidol 250 ng did not further affect spatial navigation as compared to the saline-injected group (fig 3, no overall significant difference between the two groups) However, some attention needs to be paid to the result of the third block (see fig 7, panel b) To discuss this effect, the outcome of our study on the nucleus accumbens, in which the procedure was identical to that in the present one, has to be recalled

Figure 7 (panel a) shows that *intra-accumbens* haloperidol (100 ng) had its largest inhibiting effect on the third and fourth day (Tukey test $p<0.01$ on both blocks, from [19]) So, given the postulated function of the nucleus accumbens in switching to cue-directed behaviours, this means that external cues strongly controlled the animal's behaviour (directing it to the hidden platform) at this time and that the animal heavily relied on an activated accumbens At the same point of time, haloperidol in the *dorsal striatum* tended to improve the performance in comparison to the saline-group (fig 7 b, Tukey test $p=0.098$) Such a behavioural improvement becomes understandable in view of the fact that dopaminergic antagonists applied to the dorsal striatum are known to be accompanied by a relative dopaminergic hyperactivity in the nucleus accumbens[8] This latter relative hyperactivity would then be responsible for the behavioural facilitation seen after haloperidol in the dorsal striatum in the third block A similar phenomenon was seen in a study by Jaspers et al [12] The opposite effects of haloperidol in the accumbens and dorsal striatum respectively on performance in the Morris water maze, at the same point of training, underline the differential behavioural functions of dopamine in these brain areas

As mentioned above, higher doses of haloperidol (375 and 500 ng) affect performance in both the non-spatial and spatial version of the Morris water maze Lesions of the dorsal striatum produce similar effects[28] Apparently, neither the effects of high doses of haloperidol nor those of lesions can be separated into effects on spatial localization abilities and effects on sensorimotor capacities One explanation might be that these treatments also affect the first order output station of the dorsal striatum, i e the substantia nigra (pars reticulata) This region is shown to play a role in the processing of intrinsic, proprioceptive stimuli[9], disturbed processing of these stimuli will lead to impairments in motoric behaviour

In well-trained animals inhibition of dopaminergic activity in the dorsal striatum did not disturb spatial navigation We conclude that the dorsal striatum is not active anymore during retrieval, because disturbances of its functions by means of an antagonist were no longer effective A similar phenomenon was found in the case of intra-accumbens manipulations[19]

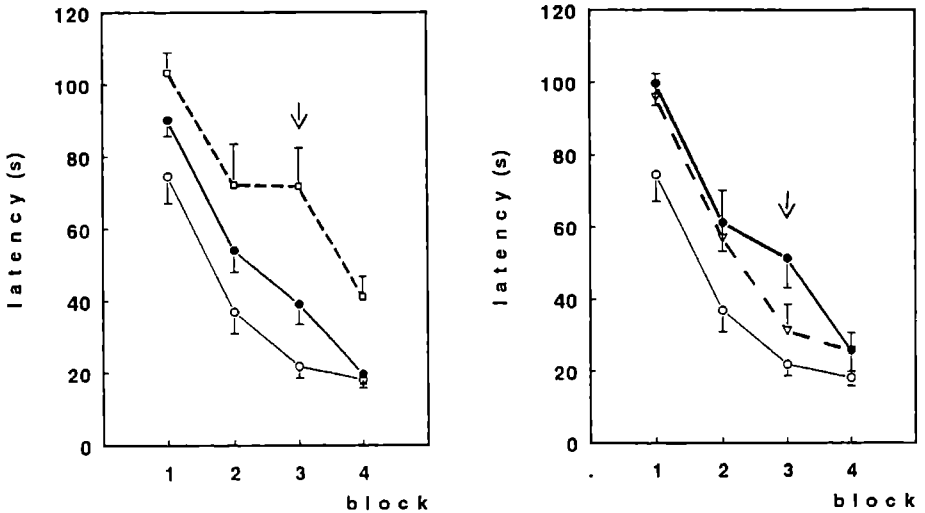

Figure 7 Comparison among the effects of haloperidol into the nucleus accumbens (left panel, non-treated group O saline-injected ●, haloperidol 100 ng □) and into the neostriatum respectively (right panel, non-treated group O, saline-injected ●, haloperidol 250 ng ▽) Attention should be paid to the third block after haloperidol 100 ng in the nucleus accumbens an enhancement in latency is seen while after haloperidol 250 ng in the dorsal striatum latency is decreased (see text)

In the egocentric localization task, performance was tested at doses below the ones that affect escaping onto a visible platform in the Morris maze Also in this paradigm, an effect of cannulation and injection was found Haloperidol 250 ng did not further impair performance This is similar to the findings in the Morris water maze (invisible platform, see above) The same explanation as given above applies here A slight, but not significant, effect was seen after haloperidol 325 ng A clearer effect might be expected at higher doses but in that case we would ascribe this at least in part to impaired sensorimotor abilities Thus, in the T-maze test used here, no specific effect of a reduced dopaminergic activity in the dorsal striatum could be established

Previous studies[6 15 20 21] have shown impairment of egocentric behaviour in radial or T-mazes after dorsal striatal lesions, but also in these studies the large lesions could have induced inhibition of the first order output station of the dorsal striatum, leading to the impairments found Furthermore, it might be that a non-dopaminergic neurotransmitter-system is taking part in the egocentric localization of a distinct goal Finally, it cannot be excluded that dopamine is active in egocentric localization in a more complex task (e g in a radial arm maze, with more choice arms[6], see also [22])

In summary, this study shows that blockade of the dopaminergic transmission in the dorsal striatum produces only deficits in allocentric spatial localization, when sensorimotor deficits co-occur No deficits were seen in egocentric spatial localization, in a simple water T-maze

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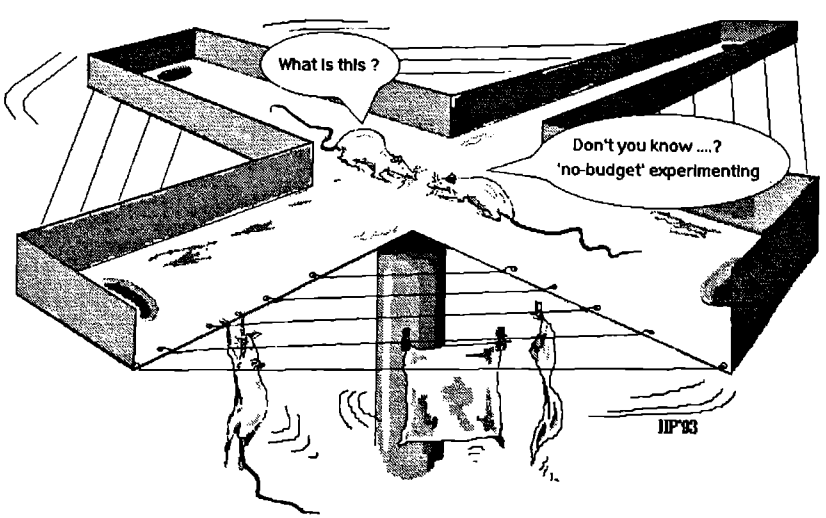
PART III: SPATIAL LOCALIZATION: RADIAL ARM MAZE



CHAPTER 6.

PERFORMANCE OF WELL-TRAINED RATS IN A SIMPLE RADIAL ARM MAZE: LACK OF EFFECTS ON RETRIEVAL AFTER MANIPULATION OF THE DOPAMINERGIC ACTIVITY IN THE VENTRAL OR DORSAL STRIATUM

G E Ploeger, L Lubbers, B M Spruijt and A R Cools

Submitted

ABSTRACT

The involvement of the dopaminergic activity in the ventral and dorsal striatum on the retrieval of a learned response in a simple radial arm maze was investigated Animals first received sufficient training in collecting a food pellet from each of four arms Thereafter and prior to the test trial, dopaminergic agonists and antagonists of two types of dopamine receptors (D2 and DA_1 respectively) were locally applied into these structures Only doses which are known to produce behavioural changes without inducing alterations in motor behaviour, were tested To stimulate and inhibit respectively the dopaminergic activity in both structures simultaneously, combined systemic and intracerebral injections were given None of these manipulations significantly altered the learned behaviour This indicates that the dopaminergic activity in these striatal areas is not involved in the retrieval of information necessary for good performance in a simple radial maze task

INTRODUCTION

Many studies report that the acquisition of various tasks is affected by manipulation of the dopaminergic (DAergic) activity For example, it has been demonstrated that systemic administration of DAergic agents affects the development of conditioned reward (Beninger, 1989, Hoffman and Beninger, 1985) Furthermore, the main DAergic structures, i e the ventral striatum, especially the nucleus accumbens, and the dorsal striatum, have been shown to be involved in learning behaviour in e g the Morris water maze (Ploeger *et al*, 1994a, Sutherland and Rodriguez, 1989) and in conditioned avoidance responding (Blackburn and Phillips, 1989, Winocur, 1974) respectively (see further for surveys Beninger, 1983, Beninger, 1989, Divac and Öberg, 1979, Phillips and Carr, 1987, Salamone, 1992)

In a previous study we have reported about the role of the DAergic activity in the ventral striatum in the acquisition of a simple radial arm maze (Cools *et al*, 1993) Animals were trained to collect a food pellet from one of four radiating arms, the DA D2-antagonist sulpiride, injected in the ventral striatum, attenuated learning of this task Likewise, DA in the dorsal striatum appeared to be active during this learning task (Ploeger *et al*, 1994c) Besides the apparent involvement in acquisition, it is questioned whether DA also plays a role in processes like retention and retrieval of information

Thus, we investigated a possible role for DA in the retrieval of information in animals, pretrained in the above mentioned radial maze, during one test trial Different DA-receptor subtypes were regarded in the ventral as well as the dorsal striatum

First, within the ventral striatum the presence of the so-called DA_1- (Cools, 1978, Cools, 1986) and the D2-receptors (White and Wang, 1986, Yang and Mogenson, 1987) has been demonstrated The activity at each of these receptors was manipulated by means of local application of the following DAergic agents With respect to the DA_1-receptor the agonist (3,4-dihydroxyphenylimino)-2-imidazoline (DPI) and the antagonist ergometrine (Cools, 1978, Cools and Oosterloo, 1983) were used Earlier, these agents have been found to specifically affect behaviour when injected in the ventral striatum (Cools *et al*, 1988, Ploeger *et al*, 1991) For manipulation of the D2-receptor, the D2-antagonist sulpiride was chosen We decided to disregard the use of specific D1-agents in the ventral striatum, because such agents (SKF 38393 D1 agonist, SCH 23390 D1 antagonist) have not been found to be behaviourally effective in a specific manner when injected into the ventral striatum (see Cools *et al*, 1988)

In the case of the dorsal striatum DA D1/D2-activity was affected by means of local injection of the well-known DAergic agonist apomorphine and antagonist haloperidol

Last, since it has been suggested that changes in the DAergic activity in the ventral striatum have consequences for the DAergic activity in the dorsal striatum and vice versa (Cools, 1980b, see also Cools and van Rossum, 1980), it was decided to study the effects of simultaneous stimulation respectively inhibition of the DAergic activity in these structures Instead of equipping the animals with four cannulas, which might have produced too much brain damage, the DAergic activity in the dorsal striatum was manipulated systemically (with apomorphine and haloperidol), whereas the DAergic activity in the ventral striatum was

manipulated by local administration (with DPI and ergometrine) (see further at the Methods) The systemically administered doses were based on previous studies In these studies, doses have been found that selectively affect the DAergic activity in the dorsal striatum (Cools, 1980a)

All of the above mentioned drugs have been shown to be behaviourally active in their respective target areas and the doses used (see below) are based on information from previous studies (Cools, 1980a, Cools and Jongen-Relo, 1991, Ploeger *et al*, 1991) Animals were trained to efficiently collect one food pellet from each of four radiating arms, then, they received an injection of one of the above dopaminergic agents, just prior to the test trial

MATERIALS & METHODS

Animals and surgery

Male Wistar rats, bred and reared in the Central Animal Laboratory, University of Nijmegen, The Netherlands, were used, weighing 180-220 g at the time of surgery After the operation, they were housed individually and maintained in a temperature and light-controlled room (lights on between 08 00-20 00 h)

For implantation of the cannulas the rats were anaesthetized with pentobarbital (Narcovet®, 60 mg/kg i p) and placed in a stereotaxic apparatus Stainless steel cannulas were bilaterally implanted, aiming at the ventral or dorsal striatum Coordinates for the ventral striatum were A= 9 8, L= +/-1 2, H=2 7, and the cannulas (length 5 mm) were inserted under a lateral angle of 10° For the dorsal striatum cannulas with a length of 4 mm were used, at the coordinates A=9 4, L= ±2 5 Locations were based on the atlas of Konig and Klippel (1963) The cannulas were then fixed onto the skull with stainless steel retaining screws and dental cement (Durelon®, Espe, Germany) (Bos and Cools, 1989) After surgery, the animals were allowed to recover for a period of at least 10 days

Three days before starting the experiment, the animals were deprived of food while water was available ad libitum During the experiment, body weight was kept constant at 75-80% of the pre-test value All experiments were carried out between 09 00 and 17 00 h Per treatment, a group of 8-10 animals was used

After finishing the experiments, the animals were sacrificed and the brains were removed and fixated with 4% formalin The precise location of the cannulas and injection sites were determined in serial sections Only the animals with injection sites within the target areas were included in the data analysis

Injection procedure and drugs

Injections were given bilaterally (as has been described elsewhere (Cools *et al*, 1993)), using a Hamilton syringe, with the needle extending 1 5 mm below the tip of the cannula for reaching the dorsal striatum or 2 mm for injecting into the ventral striatum A volume of 0 5 µl per side was delivered over a period of 10 s whereafter the needle was kept in place for another 10 s

Injection was given once, in well-trained animals, either 1, 15 or 60 min (depending on the drug injected) before the test trial (see below, experimental procedure)

In four groups the activity in the two areas was altered simultaneously by means of an intracerebral injection in the ventral striatum combined with a systemic injection of a dopaminergic agent in a dose that specifically altered the dopaminergic activity in the dorsal striatum (see Cools, 1980a) The systemic injection was applied either 30 or 3 min prior to the test trial (depending on the drug injected)

The following drugs were tested the DA_1 agonist DPI (Boehringer Ingelheim) and the DA_1 antagonist ergometrine (Sigma), the dopaminergic D2 antagonist (±) sulpiride (Sigma), the dopaminergic agonist apomorphine (ACF chemiefarma, The Netherlands) and the dopaminergic antagonist haloperidol (Janssen Pharmaceutica, The Netherlands) The drugs were dissolved in distilled water, except for sulpiride Sulpiride was dissolved in 1 25 acetic acid and the pH was adjusted with $NaHCO_3$ to pH=6-7 Injections with the solvent alone served as controls

Table 1 Summary of all the given treatments (12 in total)

TREATMENT		AGENT AND DOSE		INJECTION -TIME	n
ACCUMBENS					
no	1	aqua dest		- 15	10
	2	sulpiride	1 ng	- 15	8
	3	aqua dest		- 60	10
		(2 injections)		- 1	
	4	ergometrine	100 ng	- 60	10
	5	DPI	500 ng	- 1	10
NEOSTRIATUM					
	6	aqua dest		- 1	9
	7	haloperidol	250 ng	- 15	9
	8	apomorphine	300 ng	- 1	10
ACCUMBENS AND NEO-STRIATUM					
	9	aqua dest +		- 15	10
		apomorphine 0 05 mg/kg		- 3	
	10	DPI 500 ng +		- 15	10
		apomorphine 0 05 mg/kg		- 3	
	11	aqua dest +		- 60	10
		haloperidol 0 05 mg/kg		- 30	
	12	ergometrine	100 ng +	- 60	10
		haloperidol 0 05 mg/kg		- 30	

Footnotes to table 1

[1] Treatment 3 (2 control-injections on 60 respectively 1 minute(s) before test trial) simultaneously serves as a control for treatment 4 and 5

[2] Treatments 9-12 each consist of two injections one systemic, acting predominantly on the neostriatal dopaminergic activity and one local, into the nucleus accumbens (of the ventral striatum) (see text)

Table 2 Mean numbers of errors (± s e m) in the test trial in well-trained animals after each of 12 differen treatments (see table 1)

TREATMENT		NUMBER OF ERRORS mean ± s e m	
ACCUMBENS			
no	1	0 1	0 1
	2	0 1	0 1
	3	0 3	0 1
	4	0 2	0 1
	5	0 1	0 1
NEOSTRIATUM			
no	6	1 1	1 1
	7	0 1	0 1
	8	0 7	0 5
ACCUMBENS AND NEOSTRIATUM			
no	9	0 3	0 2
	10	0	-
	11	0	-
	12	0 1	0 1

Radial arm maze: apparatus and procedure

A four-arm radial maze was used, which has been described in detail elsewhere (Cools *et al*, 1993) In short, the maze consisted of a central hube, 34 cm in diameter, with four arms (86 cm x 9 cm) radiating from it A plastic foodwell, 1 5 cm deep and 1 cm in diameter, was located at the end of each arm The maze was constructed primarly of black plexiglass The sidewalls of the arms sloped from a height of 10 cm at the center of the maze to a height of 6 cm at the distal end of each arm Pieces of clear plexiglass extended the height of the walls to 20 cm The maze was kept in a constant position The environment could be perceived from the apparatus by the animals and contained four specific cues, one at each wall of the room in a direct line with each one of the arms of the maze

In the experiments all arms were baited with a 45 mg food pellet (Campdon Instrumental Ltd) in each trial and the animals were trained to collect all four pellets Per day they received 2 blocks, each consisting of 3 trials (one morning and one afternoon session) The rat was placed in the central hube with its head always oriented in the same direction Each trial was terminated after 10 min or after the rat had eaten all four rewards Between trials animals remained in their homecages Before each trial the maze was cleaned, the arms rebaited and then the next rat was put in the central hube When all animals had received trial 1, the second trial was given (and so on)

Each animal was trained for 6 consecutive days On the morning of the 7th day 3 criterion-trials were given only the animals that succesfully and without errors (see below for definition) completed these trials, were included in the test trial during the afternoon session Thus, in the test trial, the effects of manipulation of the dopaminergic activity on radial maze performance in well-trained animals were investigated For this purpose animals received injection(s), a

(drug)specific period of time before the test trial. Ten animals per group and twelve groups were used in total. Each of these groups received one of twelve treatments, as scheduled in table 1. Per area the applied drugs together with dosage and injection time (in min; test trial is on t=0) are stated. The control is always presented first, followed by the appropriate experimental agent(s). Furthermore, the number of animals per group is given.

First, activity in either the ventral striatum or the dorsal striatum was altered by local application of an agonist or an antagonist. Second, experiments were performed in which dopaminergic activity was either stimulated or inhibited in both areas simultaneously. Therefore, the dopaminergic agents apomorphine and haloperidol were systemically (s.c. and i.p. respectively) injected, in doses that specifically stimulated or inhibited respectively the dopaminergic activity in the dorsal striatum (see Cools, 1980a), whereas the ventral striatal dopaminergic activity was manipulated simultaneously and in the same direction by means of a local injection.

As already stated, each drug was applicated a specific period of time before the test trial. So, ergometrine was administered 1 h before the test trial because the inhibitory action of ergometrine at the level of the DA_i receptor is known to start after a delay of 45-60 min (Cools, 1978), whereas DPI was administered 1 min before the test because of its immediate effect (Cools, 1978). In the case of sulpiride a periode of 15 min was taken (Cools and Jongen-Relo, 1991). Last, haloperidol and apomorphine were applied 15 and 1 min respectively in the case of local application whereas they were administered 30 and 3 min respectively in the case of systemic application, all prior to the test trial. In the used doses they all are behaviourally effective without inducing alterations in motor behaviour (see Cools, 1978; Cools, 1980a; Cools and Jongen-Relo, 1991; Cools *et al.*, 1993; Ploeger *et al.*, 1991).

Four parameters were scored. First, *total time*, that is the time from start to end of the trial (at maximum 10 min), was recorded. Furthermore, the *collecting time* was measured, being the time between eating the first and the last (fourth) food pellet. Time from the start of the trial

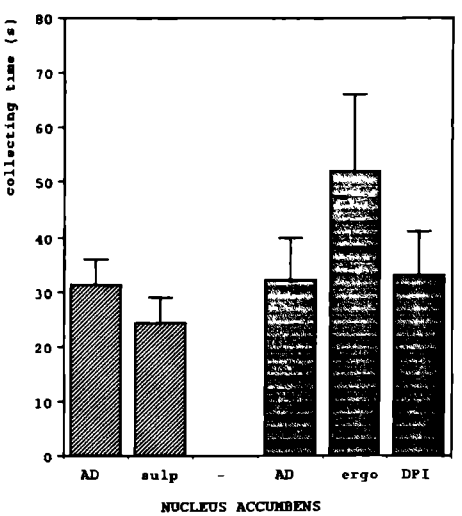


Figure 1. Collecting time (mean ± s.e.m.) on the test trial after injection of one of the dopaminergic agents sulpiride, ergometrine and DPI in the *ventral striatum* (nucleus accumbens) in well-trained rats. See table 1 for doses of the drugs and the number of animals per group.

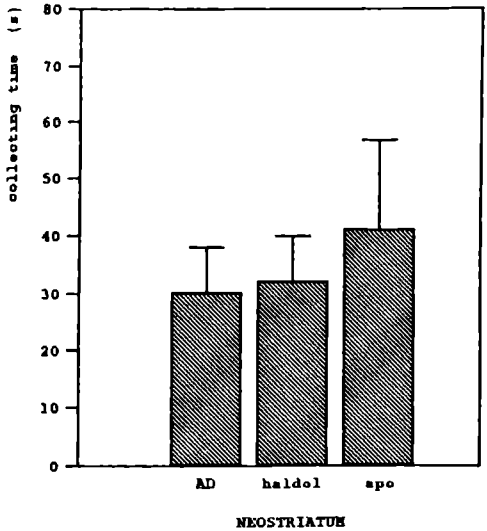


Figure 2. Collecting time (mean ± s e m) on the test trial after injection of haloperidol or apomorphine in the dorsal (or neo-) striatum in well-trained rats See further table 1

until eating the first pellet was called *latency* (summation of latency and collecting time gives total time) Last, the number of *errors* were counted visiting an arm without eating the pellet or revisiting an originally baited but emptied arm were scored as errors

Per group means ± s e m were calculated over these values For statistical analysis the non-parametric test Mann-Whitney *U* rank order test was used to compare the effect of the experimental drug with the effect of its solvent

RESULTS

All animals were checked for correct placement of the injections Four animals in total had to be excluded, in table 1 the final number of animals per experimental group is represented For an overview of the boundaries of the two target areas, the reader is referred to previous publications (e g Cools and Jongen-Relo, 1991)

During the training period of 6 days the animals acquired good and stable performance To reach the criterium, by which an animal was included in the test trial, it had to perform the last 3 trials (before testing) without errors Only one animal had to be excluded because it did not meet this criterium In an earlier study an example of performance during learning can be found (fig 1 in Cools *et al*, 1993)

In table 2 and figures 1-3 results of the post-training tests are represented

Table 2 enumerates the mean number of errors (± s e m) as displayed by each group Very few errors were made at the test trial by all groups and no differences were found between the several control and their respective experimental groups

Figures 1, 2 and 3 show the collecting time (mean ± s e m) for the different experiments Also with respect to this parameter statistical analysis showed that control values were not changed by the dopaminergic manipulation For example, in the case of injections in the ventral striatum, ergometrine did not significantly enhance the collecting time as compared to the solvent (fig 1, MW*U* test p=0 235) Likewise, in the case of simultaneous manipulation of the

ventral and dorsal striatum (fig. 3), the control groups did not differ from each other (AD/apomorphine versus AD/haloperidol, MW*U* test: n s.) and the drugs injected in the ventral striatum had no effect on performance in comparison to these control groups.
Last, the remaining two parameters (total time and latency) revealed a similar picture and did not show any significant differences either (data not shown).

Discussion

The present data clearly show that neither stimulation nor inhibition of the dopaminergic activity in either the ventral or dorsal striatum altered radial arm maze performance in well-trained animals in a one-trial test. Also the simultaneous stimulation or inhibition of both structures did not change the animal's behaviour. Thus, striatal dopamine is not essential anymore for the retrieval of learned information in a simple radial maze task.
These results are in line with the results from our previous experiments on the role of striatal dopaminergic activity in spatial localization in the Morris water maze (Ploeger *et al.*, 1994a; Ploeger *et al.*, 1994b). The escape behaviour of pretrained animals was not affected by a rather high dose of the dopaminergic antagonist haloperidol, injected either in the ventral or dorsal striatum, over three consecutive test trials. So, in both tests striatal manipulation of the dopaminergic activity only affects performance in the initial, acquisition phase (Cools *et al.*, 1993; Ploeger *et al.*, 1991).
These results are also in line with studies from the literature, reporting that dopamine does not affect retention performance of a learned response. For example, previous studies have shown that systemic and local injections of DAergic agents do not affect Morris water maze behaviour in well-trained animals (Scheel-Krüger 1992; Scheel-Krüger et al 1990). Furthermore, it has been shown that the neurolepticum pimozide in a dose that blocks the acquisition of a conditioned, environment-specific locomotor response, is unable to disrupt the expression of this conditioned response (Beninger and Hahn, 1983). Pimozide does also not

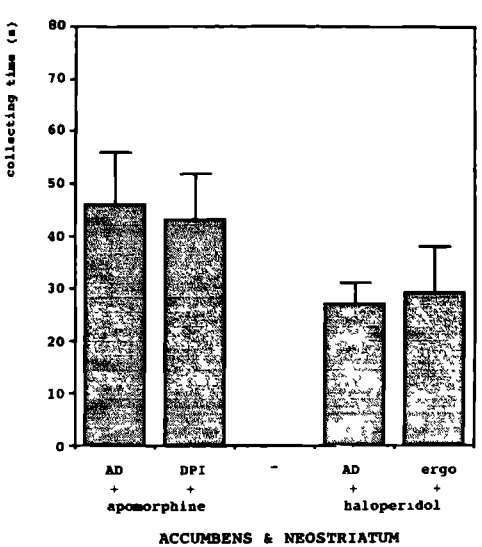


Figure 3. Collecting time (mean ± s.e.m.) on the test trial after simultaneous manipulation of the *ventral and dorsal striatum* (see also table 1 and text for more information on the treatments).

reduce lever press responding on a DRL-schedule over several test trials (Mason *et al*, 1980) Finally, neuroleptics do not disturb the expression of avoidance behaviour on the first test day (of 10 test trials) (Blackburn and Phillips, 1989) (but see also below) On the other hand however, it also appears that in certain cases dopamine is needed to maintain a learned response over a longer period of time (several trials or several days) For example, avoidance responding (Beninger *et al*, 1983, Blackburn and Phillips, 1989) as well as responding for drug self-administration (Wise and Bozarth, 1981) are inhibited by dopaminergic blockade over several test days Animals, well-trained on positively-reinforced lever press responding (CR-schedule), show a progressive decline in responding when given a neuroleptic drug (Mason *et al*, 1980, Wise and Schwartz, 1981, Wise *et al*, 1978)

Thus, it is claimed by some authors that the integrity of basal forebrain dopamine is required for the maintenance of learned responses over a longer period of time, even though some of these responses can be well-performed for some time in spite of low dopaminergic activity (see Beninger, 1983, Blackburn *et al*, 1992) For diverse experimental situations then, it needs to be examined separately to what extent dopamine function is contributing to the retention and retrieval of the learned response in question and its maintenance over a longer period of time

In conclusion, this study shows that retrieval in a one-trial test does not depend on normal dopaminergic activity, neither mesolimbic nor nigrostriatal Whether or not this independency will extend over a longer period of time is subject for further investigation

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CHAPTER 7.

ROLE OF THE DORSAL STRIATUM IN THE ACQUISITION OF A SIMPLE RADIAL ARM MAZE: EFFECTS OF LOCALLY APPLIED HALOPERIDOL IN RATS

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Submitted

ABSTRACT

The effects of the dopaminergic antagonist haloperidol, injected in the dorsal striatum, on the acquisition of a simple radial maze task were investigated Animals were trained to collect a food pellet from each of four arms Several specific environmental cues were clearly visible from the maze Prior to every day's training block they were locally injected with haloperidol, in a dose of 100 or 200 ng per side Haloperidol dose-dependently affected some of the parameters measuring acquisition, while it neither impaired motor behaviour nor increased the number of errors made The results are discussed in relation to the function of the dorsal striatum in non-cue directed (arbitrarily) switching of ongoing behaviour

INTRODUCTION

Recently it has been proposed (see Oades, 1985) that striatal dopamine plays a role in the ability of an animal to switch its ongoing behaviour Previous studies from our laboratory have established differential effects of dorsal and ventral striatal dopamine on this ability Dopaminergic activity in the dorsal striatum appears to affect switching of behaviour directed by factors intrinsic to the animal (non-cue directed) (Bercken and Cools, 1982, Vrijmoed-De Vries and Cools, 1986) On the other hand, increased dopaminergic activity in the ventral striatum (especially the nucleus accumbens) enhances the display of different behavioural items guided by external available cues (Bos, 1991, Bos *et al*, 1991)

These differential roles apply to motor and social behaviour, in rats, cats and monkeys (and for several neurotransmitter-systems) (Bercken and Cools, 1982, Cools, 1980, Jaspers *et al*, 1984, Jaspers *et al*, 1990, Vrijmoed-De Vries, 1985, Vrijmoed-De Vries, 1986) As also effects of striatal dopamine on learning and memory have been reported (see for reviews a o Beninger, 1983, Phillips and Carr, 1987, Salamone, 1992), we questioned whether this distinction is also applicable for the involvement of the striatal areas in learning and memory processes

Previous studies have provided evidence that radial arm maze performance can be altered by manipulations of the ventral (Schacter *et al*, 1989) or dorsal striatum (Colombo *et al*, 1989) Thus, we have chosen to examine a possible differential function of the striatal areas in learning and memory in a simple, basic radial arm maze procedure The rat is expected to collect one food pellet from each of four arms Several environmental cues are clearly visible for the animal in the maze

In this paradigm we may differentiate between externally structured behaviours (directed by environmental cues) and self-generated behaviours in the performance of the rat

Environmental cues indeed guide the animals, as indicated by the finding that reducing the salience of cues around the maze can change performance substantially (Mazmanian and Roberts, 1983) So, animals often re-enter previously visited arms under the condition of restricted viewing of the environment, whereas they perform more accurately when allowed to have a good look

However, only factors intrinsic to the animal determine the appearance of several other behaviours These include starting to visit arms and collect food pellets and developing a consistent way of collecting all the pellets

In view of the above mentioned differential functions of striatal dopamine, we propose that dopamine in the ventral striatum mediates the display of the externally structured strategies, while dopamine in the dorsal striatum mediates the display of internally structured (self-generated) strategies

Experiments from our laboratory (Cools *et al*, 1993) have demonstrated that dopamine in the ventral striatum, especially the nucleus accumbens, is indeed involved in the acquisition of this task Since local injection of the dopaminergic D2 antagonist sulpiride significantly attenuated radial maze learning by enhancing specifically the number of revisits, it appeared that

especially the ability to display externally structured learning strategies was affected This notion is underscored by the previous finding that the nucleus accumbens is involved in the acquisition of allocentric spatial localization in the Morris water maze (Ploeger *et al*, 1994a), in which animals solely depend on external cues to learn the task

The present study analyses the role of dopamine in the dorsal striatum in the above mentioned radial arm maze task The procedure was similar to that used in the study on the role of dopamine in the ventral striatum (Cools *et al*, 1993) Local application of the well-known dopaminergic antagonist haloperidol was used to reduce dorsal striatal dopaminergic activity

MATERIALS AND METHODS

Animals and surgery

Male Wistar rats, bred and reared in the Central Animal Laboratory, University of Nijmegen, The Netherlands, were used, weighing 180-220 g at the time of surgery After the operation, they were housed individually and maintained in a temperature and light-controlled room (lights on between 08 00-20 00 h)

For implantation of the cannulas the rats were anaesthetized with pentobarbital (Narcovet®, 60 mg/kg i p) and placed in a stereotaxic apparatus Stainless steel cannulas were bilaterally implanted, aiming at the neostriatum Cannulas with a length of 4 mm were implanted at the coordinates A=9 4, L= ±2 5 Location was based on the atlas of Konig and Klippel (1963) The cannulas were fixed onto the skull with stainless steel retaining screws and dental cement (Durelon®, Espe, Germany) (Bos and Cools, 1989) After surgery, the animals were allowed to recover for a period of at least 10 days

Three days before starting the experiment the animals were deprived of food while water was available ad libitum During the experiment, body weight was kept constant at 75-80% of the pre-test value All experiments were carried out between 09 00 and 17 00 h A group of 10 animals was used per treatment

After finishing the experiments the animals were sacrificed and the brains were removed and fixated with 4% formalin The precise location of the cannulas and injection sites were determined in serial sections Only the animals with injection sites within the target areas were included in the data analysis

Injection procedure and drugs

Injections were given bilaterally (as has been described in detail previously by Cools *et al*, 1993), using a Hamilton syringe, with the needle extending 1 5 mm below the tip of the cannula A volume of 0 5 µl per side was delivered over a 10 s-period whereafter the needle was kept in place for another 10 s Injections were given daily, 15 min before the first trial

The dopaminergic antagonist haloperidol (using a stock solution (5 mg/ml) from Janssen Pharmaceutica, The Netherlands) was dissolved in distilled water An injection with the solvent alone served as a control

Table 1 Total number of animals and the number of rats that met the first criterion (of good performance, se text) per group * p≤0 05, Chi-square test

group	number of animals	
	total	criterion
control (AD)	9	9
haloperidol 100 ng	8	6
haloperidol 200 ng	10	6 *

Radial arm maze: apparatus and procedure

A four-arm radial maze was used, which has been described in detail elsewhere (Cools *et al*, 1993) In short, the maze consisted of a central hube, 34 cm in diameter, with four arms (86 cm x 9 cm) radiating from it A plastic foodwell, 1 5 cm deep and 1 cm in diameter, was located at the end of each arm The maze was constructed primarly of black plexiglass The sidewalls of the arm sloped from a height of 10 cm at the center of the maze to a height of 6 cm at the distal end of each arm Pieces of clear plexiglass extended the height of the walls to 20 cm The maze was kept in a constant position The environment could be perceived from the apparatus by the animals and consisted of four specific cues, one at each wall of the room in a direct line with each one of the arms of the maze

In the experiments all arms were baited with a 45 mg food pellet (Campdon Instrumental Ltd) in each trial and the animals were trained to collect all four pellets Per day each animal received 1 block of 3 trials The rat was placed in the central hube with its head always oriented in the same direction Each trial was terminated after 10 min or after the rat had eaten all four rewards During the intertrial interval of 2 min the animal stayed in its homecage Before each trial the maze was cleaned, the arms rebaited, and then the animal was put in the central hube for the second trial When the animal had received all three trials, the next animal was injected and trained (and so on)

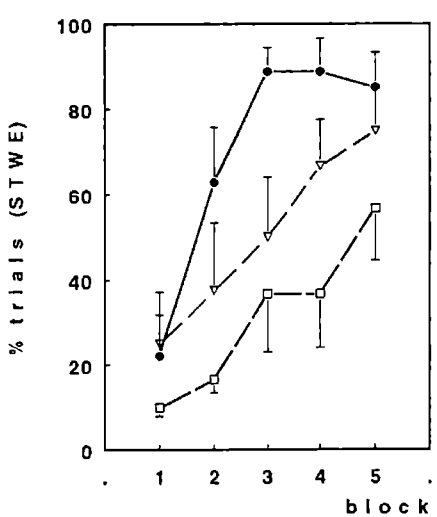

Figure 1. Mean percentage (± s e m) of successful trials without errors for water- and haloperidol-treated animals Haloperidol significantly decreased the percentage STWE, see text ● control, ▽ haloperidol 100 ng, □haloperidol 200 ng

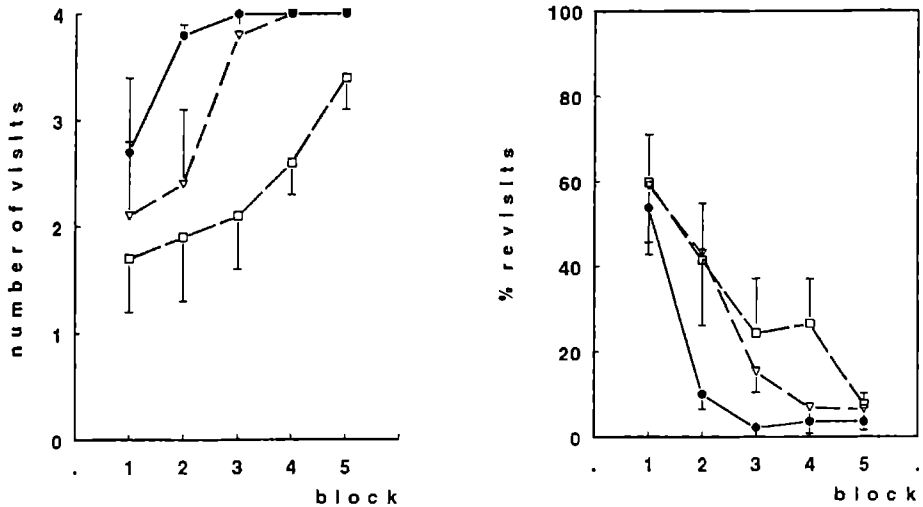

Figure 2 In the left panel (a) mean total number of visits over all trials per group is depicted, while in the right one (b) the percentage of revisits over all trials per group is shown Both parameters were significantly altered by haloperidol For both graphs ● control, n=9, ▽ haloperidol 100 ng n=8, □ haloperidol 200 ng, n=10

Each animal was trained for 5 days It received an injection with haloperidol, always 15 min before the first trial Two doses were tested 100 and 200 ng

Parameters and statistics

Several parameters were scored First *total time*, that is the time from start to end of the trial (at maximum 10 min), was recorded Furthermore, the *collecting time* was measured, being the time between eating the first and the last (fourth) food pellet Time from the start of the trial until eating the first pellet was called *latency* (summation of latency and collecting time gives, naturally, total time)

In order to have an arm entry scored, all four paws had to be placed into the arm Entering a baited arm and eating the food pellet from it was called a *visit*, while a *revisit* meant re-entering an emptied arm When the rat had entered a baited arm and had left it without eating the pellet, an *eating error* was recorded Numbers of eating errors, visits and revisits were regarded

A trial was *successful* in case the animal had collected all four food pellets, a trial in which the rat had made no revisits, was classified as *successful without errors* Both kinds of trials were counted separately ("successful trial"= ST and "successful trial without errors"= STWE)

Per treatment and per block (day) means ± s e m were calculated, for each of these recorded values This was done for all given trials, but also separately for ST respectively STWE (where appropriate)

Furthermore, two *criteria* were applied in order to evaluate the performance of the animals First, an animal was considered to perform well in the case it reached the criterion of solving three consecutive trials successfully and without errors Second, performance of the animals

Table 2 Mean number of revisits and visits per group Two animals are disregarded in the group of haloperidol 200 ng (see text)

group	n	revisits		visits	
control (AD)	9	6 0	1 0	56 0	1 9
haloperidol 100 ng	8	5 2	1 2	49 0	4 3
haloperidol 200 ng	8	6 5	0 9	42 5	2 8 *

was described in terms of the order of arm choices In particular, the number of trials was counted in which an animal, starting at one arm, consistently moved to the adjacent arm (turning consistently either left or right), collecting all food pellets without making errors An animal was regarded as having a fixed response pattern of adjacent arm choices (FRP-AAC) in the case it solved at least 5 out of 6 trials, in block 4 and 5, in such a way

To evaluate the significance of differences between the test groups, most of the data was subjected to an univariate analysis of variance (ANOVA, using the GLM procedure of the statistical SAS package) A Student's t-test or a Chi-square test was applied to analyse the differences between two successive days within one test group or to evaluate the number of animals per group reaching a criterion

RESULTS

All animals were checked for correct placement of the injections In total 3 animals had to be excluded, see table 1 for the exact number of animals per group For the precise location of the injection-sites within the target area of the neostriatum, the reader is referred to a previous study (Ploeger *et al* , 1994b)

Figure 1 shows the percentage of successful trials without errors (STWE) for the control and the two haloperidol groups Water-treated animals improved rapidly within three days more than 80% of all trials (in block 3-5) were successful and without errors Also the two haloperidol-groups showed improvement over time (overall effect of time $F(4,96)=15\ 332$, $p<<0\ 01$) However, the two experimental groups were less successful in a dose-dependent

Table 3 Latencies for the unsuccessful trials, n represents the total number of unsuccessful trials per day for eac group

DAY	CONTROL		HALDOL 100 ng		HALDOL 200 ng	
	n	mean±sem	n	mean±sem	n	mean±sem
1	9	573 20	12	573 27	20	432 55
2	1	12 -	11	493 72	19	413 58
3			2	31 19	18	254 67
4					13	362 77
5					7	124 81

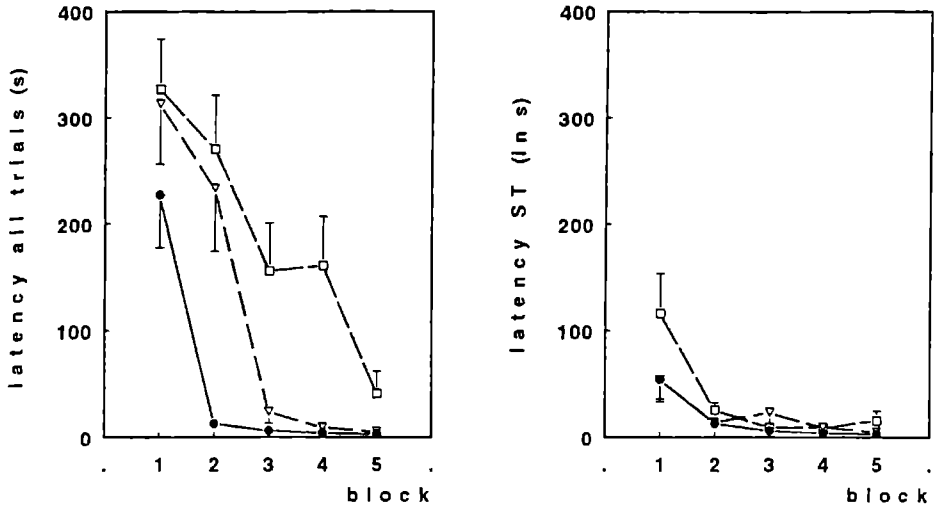

Figure 3. In these graphs latency (i e time from the start of the trial until eating the first pellet) is presented, in the left panel (a) over all trials and in the right panel (b) over the successfully finished trials For animals that successfully ended the trials, haloperidol hardly affected latency anymore ● control, ▽ haloperidol 100 ng, □ haloperidol 200 ng

manner (see figure 1), as pointed out by the significant differences in comparison to the control group ($F(2,24)=7\ 51$, $p<0\ 01$ overall effect of groups, $F(1,15)=3\ 46$, $p=0\ 076$ for haloperidol 100 ng and $F(1,17)=17\ 24$, $p<<0\ 01$ for haloperidol 200 ng)

In table 1, the number of animals per group that reached the criterion of good performance (three consecutive STWE), are represented In comparison to the water-treated group, fewer animals reached this criterion after haloperidol For haloperidol 200 ng this difference was significant Chi-square=4 29, $p\leq 0\ 05$

Performance improved also with respect to the number of visits This parameter is depicted in figure 2 (calculated over all trials) For all groups the number of visits increased over the days ($F(4,96)=13\ 9$, $p<<0\ 01$) The control rats visited the maximum number of arms already from day 3 on Both haloperidol 100 ng and 200 ng significantly decreased the number of visits as compared to the control group $F(1,245)=\ 12\ 07$, $p<<0\ 01$ and $F(1,\ 275)=75\ 36$, $p<<0\ 01$ respectively

In table 2, a summary is given of the total mean number of visits and revisits made by the different groups This table is a clear indication that haloperidol did not increase the number of revisits (errors), but decreased the number of visits For haloperidol 200 ng, this difference appeared statistically significant (Student's t-test $t=2\ 491$, $p=0\ 025$) Two animals were excluded from this latter group in this table, because these rats did not make any visit at all in about 12 out of the 15 trials and so naturally, they could not make any revisit, it is noted however that these animals made many eating errors

Latency (i e time until the animal starts to eat the first pellet) is demonstrated in figure 3 in panel a this parameter is depicted for all given trials, while in panel b only the successful trials are considered Latency decreased for all three groups, but most rapidly and to a very low level for the water-treated control group In the case latency is regarded over all trials,

Table 4 Number of animals per group with a fixed response pattern of adjacent arm choices (FRP-AAC) The number is significantly lowered after haloperidol 200 ng (Chi-square test, ** $p \leq 0.02$)

group	total number	number of FRP-AAC
control (AD)	9	7
haloperidol 100 ng	8	3
haloperidol 200 ng	10	2 **

haloperidol significantly enhanced this parameter in a dose-dependent manner, $F(1,245)=12.45$, $p<<0.01$ for haloperidol 100 ng and $F(1,275)=38.98$, $p<<0.01$ for haloperidol 200 ng Considering the successfull trials, latency was enhanced only in the first block after haloperidol 200 ng Statistical analysis revealed for this dose a significant effect of groups, $F(1,188)=6.78$, $p=0.01$ This differential effect of haloperidol on latency means that only in the unsuccessful trials latencies remained high (see table 3)

Figure 4 shows the collecting time for the control and haloperidol groups in the successful trials without errors Time needed to collect all four food pellets decreased over the blocks for all groups ($F(4,142)=10.67$, $p<<0.01$ for haloperidol 100 ng and $F(4,130)=4.11$, $p<0.01$ for haloperidol 200 ng) An effect of groups was apparent haloperidol increased the collecting time, in a dose-dependent manner ($F(1,142)=22.71$, $p<<0.01$ for haloperidol 100 ng and $F(1,130)=51.66$, $p<<0.01$ for haloperidol 200 ng)

Last, it was examined whether the animals acquired fixed response patterns while collecting the food pellets It showed that most of the water-treated rats consistently moved from one arm to the adjacent arm at the end of the training, thereby always turning one way around (either left or right) The number of animals showing such a fixed pattern (definition of the criterion see Methods) is presented in table 4 for all three groups It is clear from this table that haloperidol decreased the number of rats with a fixed response pattern of adjacent arm choices (FRP-AAC, Chi-square=6.17, $p \leq 0.02$ for haloperidol 200 ng)

Discussion

Although the time until eating the first food pellet (latency) was enhanced by haloperidol when all trials were counted, it appeared that latency was not enhanced in those trials that were successfully finished (fig 3 a and b) Only in the unsuccessful trials rats remained slow in starting to collect (table 3) Thus, the applied doses of haloperidol did not induce a general motor deficit (sedation), because such a motor disturbance would be expected to affect all trials equally

Haloperidol did also not alter the mean total number of revisits (taken over all 15 trials, see table 2), so, the experimental rats did not make more *errors* as compared to the control rats This finding means that an animal (regardless of its treatment), after having visited one arm, appears to be capable of remembering its visit to this arm, possibly because of good association with the cue nearby this specific arm This aspect of the acquisition of radial maze performance is thought to be mediated via the nucleus accumbens (see Introduction) and as the activity in this structure is not inhibited, it is understandable that this aspect is left intact

However, haloperidol affected other parameters measuring the acquisition of radial arm maze performance

After treatment with the relatively low doses of 100 and 200 ng haloperidol, animals finished fewer trials successfully and without errors (fig 1) and visited fewer arms (fig 2, table 2) as compared to control animals The effects were larger for haloperidol 200 ng than for haloperidol 100 ng (dose-dependency) Haloperidol 200 ng significantly reduced the number of animals that reached the arbitrarily chosen criterion of good performance (three consecutive successful trials without errors, table 1)

Haloperidol also increased the time needed to collect all food pellets (fig 4) and again the effect was larger for the higher dose

Furthermore, close analysis of the data considering the order of arm entries revealed that the haloperidol-treated animals failed to develop a simple and effective strategy Animals in the control group appeared to acquire a fixed and effficient way of collecting the food pellets seven out of nine water-treated animals consistently moved to the next adjacent arm, turning either left or right, at the end of the training period (criterion of FRP-AAC, see the Methods-section) This suggests that rats can, and normally will, acquire a response strategy to solve the task Most of the animals treated with haloperidol did not reach this phase (table 4)

Overall, haloperidol increased latencies in unsuccessful trials, it decreased the number of visits, it increased time needed to collect all food pellets and it prevented the development of a

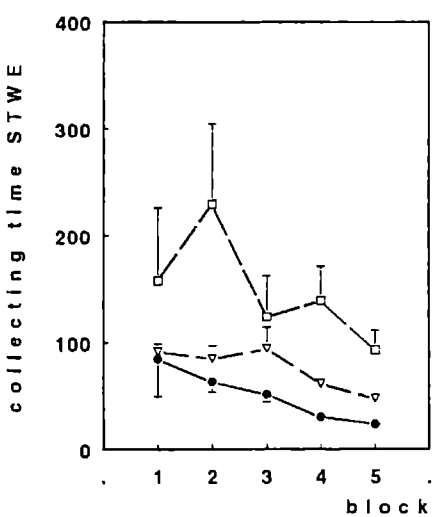

Figure 4. Mean collecting time (± s e m) over STWE per group, haloperidol enhanced this parameter ● control, ▽ haloperidol 100 ng, □ haloperidol 200 ng

consistent way of collecting all the pellets These specific effects of haloperidol, all dose-dependent, show that haloperidol-treated animals have difficulties with the arbitrarily initiation of appropriate behaviour at distinct points of time (not due to motor disturbances, see above) starting to collect, visiting a next arm or developing an effective way of collecting Only factors intrinsic to the animal determine the display of these behavioural items Thus, it is concluded that haloperidol injected in the dorsal striatum interferes with the performance in a simple radial arm maze, because it interferes with the function of the dorsal striatum in switching to non-cue directed behaviours

Altogether, we have now provided evidence that both the ventral (Cools *et al*, 1993) and dorsal (this study) striatum play their own specific role in the acquisition of simple and classic radial maze performance inhibition of ventral striatal activity attenuates the acquisition because it impairs the ability of the organism to display externally structured learning strategies, whereas inhibition of dorsal striatal activity attenuates the acquisition because it impairs the ability of the organism to display internally directed or self-generated learning strategies

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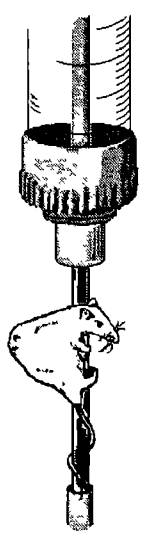
CHAPTER 8.
GENERAL DISCUSSION



In the studies described in this thesis we aimed to examine a possible differential role of ventral and dorsal striatal dopamine in cue- and non cue-directed aspects of learning and memory, respectively For that purpose, we employed three different learning and memory tasks, that enabled us to investigate the effects of specific dopaminergic agents on these different aspects

Comprehension of the role of dopamine in diverse behavioural functions is important, among others, for understanding the consequences (symptoms) of dopaminergic dysfunctioning in several human diseases involving basal ganglia disorders, such as Parkinson's disease, Huntington's chorea or schizophrenia These symptoms may include disturbances in motoric behaviour as well as in cognition (including learning and memory), depending on the kind and/or magnitude of the neuronal damage

A great deal of research is directed towards collecting clinical data concerning these diseases (describing symptoms and trying to classify them, testing preserved abilities, interpreting the obtained data) Although it is beyond the scope of the present thesis to deal with that research, awareness of the clinical practice may help to determine the direction of animal research concerning basic questions on the working mechanisms of brain areas and the involvement of neurotransmitters

As one result of the clinical research, hypotheses on the role of the basal ganglia as being involved in procedural or implicit learning and memory have been put forward (Phillips and Carr, 1987, Knopman and Nissen, 1991) However, as mentioned before in § 1 4, such distinctions appear to be insufficient in explaining all the observed deficits and preserved abilities

Thus, we have proposed an alternative view, involving a differential role for the ventral and dorsal striatal areas in externally and internally structured learning and memory, respectively, for several reasons Anatomically, the ventral and dorsal striatum appear to be part of separate neuronal circuits (roughly VS $\leftrightarrow$ limbic cortex, DS $\leftrightarrow$ neocortex, respectively, see § 1 3) Cognitive deficits following damage in each of these areas can be grouped accordingly (e g Picirilli et al , 1989) Furthermore, studies from our laboratory have already demonstrated a differential role for the ventral and dorsal striatum in externally and internally guided aspects of swimming behaviour (§ 1 4)

So, in this thesis basic animal (rat) studies were carried out to investigate whether the hypothesized distinction also applies to learning and memory processes Eventually, growing insight in the specific roles of the striatal areas in behaviour of all kinds may be useful in the description and explanation of human basal ganglia pathology

This general discussion will not focus on specific outcomes of each experiment Those outcomes are described and discussed in the previous chapters (2-7) and enumerated in the Summary The present chapter will focus on whether or not the results from the experiments

support the hypothesis on a differential role for ventral and dorsal striatal dopamine in learning and memory

Before doing so, attention is first paid to when dopaminergic treatment is most effective during the course of the learning and memory process, and second, to the choice of the applied dopaminergic agents and its implications

EFFECT OF DOPAMINERGIC TREATMENT ON DIFFERENT LEARNING AND MEMORY PHASES

In the studies in the present thesis, dopaminergic agents have been applicated at different points of time during the learning and memory process pre-training (Morris water maze (MWM), T-maze (TM) and radial arm maze (RAM), prior to a block of 3 or 4 training trials), post-training (social memory, immediately after a one-trial training), and pre-retrieval test (MWM and RAM, prior to a block of 3 or 4 trials or to one test trial, respectively)

In the Morris water maze and the radial arm maze dopaminergic treatment clearly affected performance during the acquisition, while leaving the responding during the retrieval test(s) intact Furthermore, the early application of DPI in the social memory task, immediately after the training trial, was also found to be effective In this latter case, the agents DPI and ergometrine are assumed to influence consolidation of the information, but influence on the retrieval of stored information cannot be excluded Additional experiments are required to distinguish between these two possibilities

Thus, our data point to dopamine mainly being effective in the early phases of the learning and memory process In literature, effects of dopaminergic treatment on the acquisition phase or at immediate post-training manipulation also have been reported (for instance, in avoidance responding or in lever pressing for food, see § 1 2) However, the data are not unambiguous with respect to effects on retention of acquired information or responding Inhibition of dopaminergic activity may soon interfere with the learned performance or it may take a large number of trials before the response is finally diminished under the influence of prolonged dopaminergic inhibition (Blackburn and Phillips, 1989, Wise et al , 1978, Sanger, 1986, see Blackburn et al , 1992) Overall, several authors concluded that dopamine is involved in the acquisition of specific information or responses but may also be needed to maintain the learned information over a longer period of time (see Beninger, 1983, Blackburn et al , 1992)

Generally speaking, a particular dopaminergic brain structure may seem no longer to be involved in the retention of information or the execution of a well-learned response, when inhibition of the dopaminergic activity in that area initially appears not to alter that information or response This finding may lead to the conclusion that the neuronal circuit maintaining the information or the response resides outside the dopaminergic structure However, when prolonged inhibition of the dopaminergic activity ultimately appears to result in some kind of extinction of the performance, this finding (when not due to mere motoric deficits) implicates that the neurotransmitter activity in the dopaminergic structure apparently continues to exert

influence on the neuronal circuit sustaining the information or well-learned response, either directly or indirectly

In our experiments no prolonged retention testing was performed, as only one (RAM) or 3-4 (MWM) retrieval test trials were carried out In these instances, inhibition of the dopaminergic activity did not interfere with retrieval of the learned information or the execution of the acquired response This resistance against dopaminergic interference might hold for a longer period of time, because the MWM task is a rather coercive event and because in the RAM the animals were trained for a relatively large number of trials Both these factors may strengthen the memory function, making it more resistant to decreased dopaminergic function, similar to the finding that the intensity of an applied shock in avoidance responding can protect memory function against memory impairing treatments (Pérez-Ruiz and Prado-Alcalá, 1989)

Overall, the data from our experiments showed that dopaminergic manipulation exerts its greatest influence in the early stages of the learning and memory processes Prolonged testing of well-trained animals under influence of dopaminergic agents is required to determine how long the acquired responses will resist dopaminergic treatments

USE OF DIFFERENT DOPAMINERGIC AGENTS

Several dopaminergic agents were used, acting on different dopamine receptor sub-types haloperidol and apomorphine, DPI and ergometrine, and sulpiride

In many of the experiments only haloperidol was applied This agent is well-known for being a dopaminergic (mixed D1/D2) antagonist, but is also known to act on α-noradrenergic receptors and, although to a much lesser extent, on serotonergic receptors (Burki, 1986, Richelson and Nelson, 1984)

In the *dorsal striatum*, use of haloperidol in the Morris water maze and the radial arm maze (and of the dopamine mixed D1/D2 agonist apomorphine in the RAM retrieval test) was meant to influence the dopaminergic receptors, since haloperidol (and apomorphine) was injected into the so-called dopaminergic region of the dorsal striatum (Vrijmoed-de Vries and Cools, 1986) Further experiments showing that a dopaminergic agonist (apomorphine) can attenuate the effect of haloperidol are needed to demonstrate dopaminergic specificity of the observed effects in the acquisition of the RAM, whereas application of more specific dopaminergic agents are needed to establish whether the involvement of dopamine is mediated via the D1-like or D2-like receptor subtypes (Sibley et al , 1991)

In the *nucleus accumbens*, the following neurotransmitter systems may be of importance the dopaminergic D2 and DA_i as well as the noradrenergic receptors More precisely, reports from literature indicate the presence of two separate pathways The first one encloses noradrenergic afferents from the locus coeruleus to the accumbens that may control, via ß-noradrenergic receptors, the dopaminergic D2 activity, which in turn appears to modulate the incoming signals from the hippocampus to the nucleus accumbens (see Cools et al , 1991 and cited references therein) The second pathway involves noradrenergic afferents from the ventral noradrenergic bundle to the nucleus accumbens that may control, via α-noradrenergic

receptors, the dopaminergic activity at the DA_1 receptor level, which in turn appears to modulate the incoming signals from the basolateral amygdala (see Cools et al , 1991) So, depending on the applied dopaminergic agent, different effects may be achieved

It may now become conceivable that intra-accumbens injections of haloperidol, supposed to act on the dopamine D2 receptors controlling the input from the hippocampus, are effective in spatial learning First, dopamine D2 has been demonstrated to be involved in the display of cue-directed behaviour the D2 agonist LY 171555 enhanced the number of cue-directed behavioural items in the one trial swimming task, whereas a by itself ineffective dose of the D2 antagonist raclopride was able to attenuate the effect of LY 171555 (Bos et al , 1991) Second, the hippocampus is known to be important for the acquisition and retention of allocentric spatial localization (see § 1 5) So, the effect of haloperidol injected into the nucleus accumbens on the localization of the hidden platform in the Morris water maze by use of external cues may be mediated via the dopamine D2 receptors acting on the hippocampal afferents

However, a major problem arises when data from electrophysiological studies are taken into account Enhanced dopaminergic activity at the D2 receptors is said to inhibit the hippocampal incoming signals and vice versa (Yang and Mogenson, 1986) If so, inhibition by haloperidol would enhance the incoming signals from the hippocampus, which then might be expected to result in an enhanced performance in the Morris maze This outcome is opposite to the observed effects and their explanation along the above given reasoning Assuming that haloperidol is acting on the α-noradrenergic receptors will not resolve this problem Inhibition of the noradrenergic activity is assumed to enhance the dopaminergic activity at the level of the DA_1 receptor, which in turn inhibits the signals from the amygdala As extensively discussed elsewhere, attenuated input from the amygdala is suggested to coincide with enhanced input from the hippocampus (see Cools et al , 1991) If so, this would constitute a similar situation as compared to action of haloperidol on the D2 receptor on the hippocampus afferents

Several solutions or explanations may be put forward to resolve the problem One likely possibility is the following, which was earlier proposed by Bos (see Bos, 1991) Electrophysiological experiments have shown that dopamine is able to influence the excitation of nucleus accumbens neurons induced by hippocampal stimulation, probably via the dopamine D2 receptor (De France et al , 1981 and 1985, Yang and Mogenson, 1984 and 1986) Mogenson and coworkers, applying low frequency stimulation (0 5-1 5 Hz) of the hippocampus, reported an inhibitory action of enhanced dopaminergic activity on the input from the hippocampus However, De France and coworkers demonstrated a frequency dependent effect of dopamine Dopamine inhibited hippocampus excitation of the accumbens neurons when low frequency stimulation (0 5 Hz) of the hippocampus was applied, while dopamine was ineffective at higher frequency stimulation (6 0 Hz) of the hippocampus These

findings imply that input from the hippocampus is selectively transferred to the nucleus accumbens under the influence of dopamine (De France et al , 1981 and 1985) The high frequency stimulation is within the range of the hippocampal theta rythm, an important firing pattern from the hippocampus that is related, among others, to the display of exploratory behaviour O'Keefe and Nadel (1978) hypothesized that also the construction of a spatial map is strongly related to the display of theta activity in the hippocampus

Another explanation might be that an optimal level of dopaminergic activity at the dopamine D2 receptors is needed for proper transfer of information from the hippocampus to the nucleus accumbens, which may be present in normal, untreated rats Any disturbance in this level, either by stimulation or inhibition, would then induce a disturbed performance in the Morris maze However, this reasoning would not be in agreement with the previous findings by Bos (1991) showing opposite effects of a dopaminergic D2 agonist and antagonist on the number of cue-directed behaviours in the one trial swimming test

Finally, as the interactions between dopamine and glutamate (from the hippocampal afferents) are not yet fully understood (see § 1 3), haloperidol (if mediated by dopamine D2 receptors) may be acting on D2 receptors located at different places in the local circuitry of the nucleus accumbens Future experiments will certainly have to consider questions on the specificity of the effects following haloperidol treatment, the interactions between dopamine and glutamate in the nucleus accumbens (and the dorsal striatum as well) and existing inconsistencies at cellular versus behavioural levels

The dopaminergic agents DPI and ergometrine in the social recognition task, injected into the nucleus accumbens, will be acting on the dopamine DA_1 receptors, that appear to control the amygdala input Enhancement of the dopaminergic activity at the level of the DA_1 receptor is assumed to inhibit the input from the amygdala to the accumbens, which is supposed to coincide with an enhanced input from the hippocampus (see Cools et al , 1991)

Both the hippocampus and amygdala are part of at least one of the two circuits for processing olfactory information (the main olfactory system or the accessory olfactory system, see § 1 5) It is not yet known in what way each of these two areas contribute to the process of the recognition of a juvenile conspecific Treatments within the septum, a brain structure closely linked to the hippocampus, have been reported to alter social recognition (Popik, 1991), which might imply the hippocampus in this behaviour On the other hand, the accessory or 'vomeronasal' olfactory system is known to contribute to the action of pheromones (see § 1 5), the 'vomeronasal amygdaloid structures' may thus be expected to play a role in social recognition Consideration of the anatomical basis, however, raises one problem the 'vomeronasal function' of the amygdala is related to the medial amygdaloid group whereas the projections to the nucleus accumbens derive mainly from the basolateral amygdala (see § 1 3 and Olmos et al , 1985)

Clearly, further experiments will have to consider in what way the drug-induced neurotransmitter status is related to the involvement of either of these two brain structures in social memory

One last remark must be made on the question of the dopamine specificity of the observed effects In the present thesis no antagonism experiments in the MWM and RAM learning have been described

Pilot experiments with the dopaminergic agonist apomorphine (mixed D1/D2) sofar have not yet resulted in a clear attenuation of the effects of haloperidol

Several explanations may be given First, it is difficult to establish the appropriate dose to be used Second, any manipulation of the dopaminergic activity, irrespective of the direction, might induce deterioration of the maze behaviour, in comparison to the behaviour of an untreated control animal Third, the action of haloperidol may be mediated via other neurotransmitter systems

At present, we continue to ascribe the effects of haloperidol to a dopaminergic (D2) effect, as this agent is indeed a well-known antagonist of the dopaminergic activity (also known in the clinical practice, where it is used as a neuroleptic drug) and it appears to bind relatively more strongly to dopaminergic D2 receptors than to noradrenergic or serotonergic receptors (Richelson and Nelson, 1984) However, unambiguous demonstration of a role for the dopaminergic activity in Morris maze and radial arm maze will have to be pursued

STRIATAL DOPAMINE IN CUE-DIRECTED VERSUS NON-CUE DIRECTED LEARNING AND MEMORY STRATEGIES

The main issue of this thesis is the examination of a possible differential role for ventral and dorsal striatal dopamine in cue-directed and non-cue directed aspects, respectively, of learning and memory Do the obtained data support such a differential role?

We have employed two obviously cue-dependent tasks Social recognition is based on olfactory cues emitted by the younger animal (Sawyer et al , 1984, Popik, 1991) and spatial localization of the hidden platform in the Morris maze appears to depend on extra-maze cues (Morris, 1981) The dependence on extra-maze cues in the MWM is confirmed in our control experiment in chapter 3, showing that removal of salient cues from the environment impairs spatial learning in non-treated animals

Data from our experiments sofar have demonstrated an involvement of the nucleus accumbens in these two tasks Enhancement of the dopaminergic DA_1 activity in the accumbens enhanced the memory for the juvenile conspecific, the effect appeared indeed to be dopamine specific as ergometrine was able to attenuate this effect of DPI

Inhibition of the dopaminergic activity in the accumbens induced a dose-dependent attenuation of the acquisition of the platform localization, without affecting locomotor behaviour, sensorimotor capacities needed in the MWM or the motivation to escape Although up to now

dopamine specificity has not unequivocally been established, these data point at least to an involvement for the nucleus accumbens

In addition to the demonstration of a role for the accumbens in spatial localization, the dorsal striatum, with its proposed opposite behavioural function, was shown not to be specifically involved in Morris water maze behaviour Results from the dopaminergic manipulation in the dorsal striatum in the MWM differ from the results after dopaminergic treatment in the nucleus accumbens in several respects Impairments occurred only after much higher doses Haloperidol injections into the dorsal striatum seemed to affect localization of the hidden platform in an all-or-non manner (as compared to saline injections) In addition, impairment of spatial localization appeared only to co-occur with impairment in the non-spatial task, indicating sensorimotor deficiencies Performance in the task with the visible platform did not approach control levels at the end of training

Taken together, the results from the MWM experiments with haloperidol injections into the ventral and dorsal striatum suggest, in line with our hypothesis, that the ventral striatum plays a specific role in spatial localization based on external cues, while the dorsal striatum does not

In the radial arm maze, animals can apply different strategies to solve the task They may associate each arm with its nearby cue on the wall or they may develop a specific fixed response pattern, unrelated to stimuli from the environment Normally, also spatial mapping abilities may be used However, the environment was almost empty in our experiments, except for the four specific cues on the wall, making this possibility less likely Radial arm maze behaviour can be divided into two classes Minimal one behavioural item is assumed to be directed by stimuli from the environment remembering which arm already has been visited by means of association with the nearby cue on the wall, preventing the animal from re-entering an already visited arm In contrast, only factors intrinsic to the animal may direct the animal to display several other behavioural items These include starting to visit the first arm and collecting food, visiting a next arm and developing an efficient fixed response pattern irrespective of the environment

In the present thesis inhibition of the dopaminergic activity in the dorsal striatum was shown to affect specifically these latter kinds of behaviours In a previous study (Cools et al , 1993), a role for ventral striatal dopamine in remembering already visited arms has been established

Together, the data from the RAM experiments also support a differential role for ventral and dorsal striatal dopamine in cue-directed and non-cue directed aspects of learning, respectively

Nevertheless, some difficulties remain, two of which will be discussed here

First, attention may be drawn to different effects of dopaminergic activity in different learning and memory tasks In our experiments, injections of haloperidol, in the relatively low doses of 100-200 ng into the dorsal striatum, were found to be effective in the radial arm maze Only higher doses induced effects in the spatial Morris water maze, which in addition appeared to be non-specific

It was concluded that haloperidol in the RAM acted on those behaviours that were controlled by factors intrinsic to the animal In the MWM, we also might assume intrinsically structured behaviours to be present Since external cues only gradually gain control over the rat's behaviour in directing it to the hidden platform during the course of training, the rat must arbitrarily determine what to do, especially in the beginning of the training As the Morris maze task is more difficult than the radial maze task, it might be expected that a (slight) effect of a relatively low dose of haloperidol was found on such an aspect in the MWM Different explanations may be raised for the apparent lack of a specific effect of haloperidol in the dorsal striatum on MWM performance

Latency, the only parameter reported in the studies of MWM behaviour, is probably inadequate to show a possible deficit in intrinsically structured behaviours in the MWM It may be that an effect of a relatively low dosis of haloperidol can be found on non-cue directed behaviour(s) in the MWM, when regarding an appropriate parameter, for instance the time until an animal starts to swim away from the edge of the pool

Besides, the importance of one kind of behaviour, and thus the effect of some treatment into a brain region involved in that behaviour, may naturally be greater in one experimental test than in another Furthermore, in a more coercive experimental set-up an animal may overcome its behavioural deficits and perform relatively well In sum, the magnitude of effect of a particular treatment on some kind of behavioural function depends on the nature and degree of coercion by the task to perform a response, the degree of task difficulty and the importance of the brain area in the behavioural function under investigation

It further remains difficult to explain why an effect of the cannulation and injection procedure was found in the spatial tasks and not in the non-spatial tasks, whereas an injection of haloperidol 250 ng into the dorsal striatum does not further attenuate spatial localization of the hidden platform The cannulation effect itself may be taken to indicate that the areas, through wich the cannulas are running, play a role in the observed behaviour

Second, the notion of the term 'cue' may be subject of discussion Often, the terms stimulus and cue are used interchangeably among different authors, although they are not always meant to indicate the same

In the spatial localization task, the external, distal cues may be regarded as a complex set of formerly neutral stimuli that gain meaning during the training period and acquire the ability to direct the animal to the hidden platform The nucleus accumbens is supposed to play a role in this process

The nucleus accumbens is already known to be involved in the process of secondary reinforcement (Everitt et al , 1989, Cador et al , 1991), in which a previously neutral stimulus gains meaning via association with a primary (natural) reinforcer, thus acquiring the ability to motivate the animal to respond in a particular way This process is proposed to enact within

the amygdala (known for processing distinct elements of a single stimulus (Scheel-Kruger and Willner, 1991)) and its connection to the accumbens (Cador et al , 1991)

In a similar way, we propose the nucleus accumbens to be involved in the process of encoding the meaning of a complex set of stimuli (the spatial relations between environmental stimuli) in association with an appropriate motor response (escaping), enacting within the neuronal pathway of (at least) the hippocampus and its connection to the accumbens The hippocampus has previously been demonstrated to process the relationships between external stimuli in constructing a spatial map of the environment (O'Keefe and Nadel, 1978) and, in a broader sense, to process complex sets of contextual stimuli (Sutherland et al , 1989, Sutherland and Rudy, 1989) The dopaminergic activity in the nucleus accumbens might then strengthen the association between the information on the spatial relations among external cues and the appropriate responses of approaching and escaping onto the platform, possibly because of rewarding properties of the escape from the water

In contrast, the visible platform is not regarded as a cue The visible platform acts as a clear beacon and learning to escape onto it is regarded as very straightforward the only requirement is heading towards the platform and climbing onto it The hippocampus is not involved in this response (Morris et al, 1982) Although, of course, there is a small learning component in this task (the rat has at least to learn about the procedure), it is mainly regarded as a control test for sufficient swimming abilities, detecting capacities and motivational drive to escape from the water (Morris, 1981 and 1984) The brainstem, including the colliculus superior and the basal ganglia (!), has been reported to sustain the response in the MWM with the visible platform (Whishaw and Kolb, 1984) However, more precise experiments are required to investigate which one or which part of these structures in particular is involved

Nevertheless, it is difficult to appreciate the precise difference between the visible platform in the non-spatial MWM and the stimulus (the wall of the watertank) involved in directing the rat's behaviour in the one trial swimming test, used to demonstrate a role for the accumbens in cue-directed behaviour (Bos et al , 1991, Bos, 1991) In fact, the wall-stimulus was even more proximal and may have had less meaning than the visible platform From this point of view, it is not easy to understand why the former is said to direct the animal's behaviour and the latter is not The difference may be that the former does not present the solution, whereas the latter does

With respect to the proposed function of the dorsal striatum in non-cue directed behaviour, it is stressed that this area receives a huge amount of input from many cortical areas, among which sensory cortices This fact implies, as can be understood intuitively, that behaviour controlled by (dopaminergic activity from) the dorsal striatum is not said to be without any influence from external stimuli or cues It only means that the external information reaching the dorsal striatum via the cortical sensory, motor and association areas may be much more processed, in this way being part of a complex set of 'internal factors' (considerations, motivations, experiences, recollections, etc)

OVERALL

In conclusion, the results from the experiments presented in this thesis support the hypothesis of a differential role for striatal dopamine in learning and memory the dopaminergic activity in the ventral striatum appears to play a role in learning and memory tasks based on or directed by external cues, whereas the dopaminergic activity in the dorsal striatum appears to sustain internally structured behaviours in learning and memory tasks In addition, striatal dopaminergic activity appears to be most effective in the early stages of the learning and memory process

Issues remaining to be investigated include the establishment of which of the (dopaminergic) transmitter sub-types are involved, the nature of the stimuli or cues that are capable of directing the animal's behaviour and the relationships of the nucleus accumbens with the hippocampus on the one hand and the amygdala on the other

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SUMMARY

The present thesis examined the hypothesis of a differential role for ventral and dorsal striatal dopamine in cue-directed and non cue-directed aspects of learning and memory, respectively, in three different learning and memory tasks The experiments (described in the chapters 2-7) enabled us to investigate the effects of specific dopaminergic treatments on the different aspects

SOCIAL MEMORY (CHAPTER 2)

The dopamine DA_1 agonist DPI, injected into the nucleus accumbens immediately after the training trial, significantly decreased the duration (and frequency) of social investigation of a conspecific juvenile by an adult male rat at the second exposure after a long intertrial interval This finding is interpreted as an improvement in recognition of the juvenile by the adult animal The dopamine DA_1 antagonist ergometrine, injected into the nucleus accumbens in combination with DPI at the appropriate time, was able to attenuate the effect of DPI An injection of ergometrine alone was ineffective Thus, the memory improvement appears to be dopamine specific

We have now provided evidence for a memory improving role of the dopaminergic activity at the DA_1 level within the nucleus accumbens

SPATIAL MAPPING (CHAPTERS 3-5)

An involvement of dopaminergic activity in allocentric spatial localization was indicated by effects found in the standard Morris water maze task after pre-training systemic injections of the dopaminergic antagonist haloperidol in low doses These doses impaired neither locomotion in the open field test nor learning to escape onto a visible platform in the Morris maze

Further investigations provided evidence for a specific role of the dopaminergic activity in the nucleus accumbens Acquisition of allocentric spatial localization of the platform was similarly impaired after pre-training intra-accumbens injections of haloperidol at low doses in a dose-dependent manner Again, these doses did not significantly impair locomotion in the open field or learning to escape onto a visible platform (non-spatial)

In contrast, results from haloperidol treatment in the dorsal striatum yielded a different picture Haloperidol at a low dose did not affect allocentric spatial learning, while two higher doses impaired escaping onto both the visible and invisible platform (without a dosis-response relationship in both situations) This finding suggests that dopaminergic activity in the dorsal striatum is not specifically involved in spatial localization in the Morris water maze

Good performance in the Morris water maze in well-trained rats did not deteriorate after high doses of haloperidol injected into the accumbens or into the dorsal striatum, prior to the retrieval test (of 3 or 4 trials)

The involvement of the dorsal striatum in egocentric spatial localization has been regarded in a simple water T-maze In this task (not described in the Introduction, but see chapter 5), the animal can learn to find the escape platform by consistently turning to one side, irrespective of the environment No significant effect of the chosen doses of haloperidol in the dorsal striatum was found on egocentric spatial localization in this simple T-maze

Overall, the nucleus accumbens was demonstrated to play a specific role in the acquisition of allocentric spatial learning, while the dorsal striatum only exerted a non-specific influence Whereas acquisition was affected, retrieval of the well-learned information and response was unaffected by striatal dopaminergic manipulation

RADIAL ARM MAZE BEHAVIOUR (CHAPTERS 6 AND 7)

One-trial retrieval of acquired information in rats trained to solve a simple radial arm maze task is not affected by alterations in the dopaminergic activity in the ventral or the dorsal striatum

In contrast, inhibition of dopaminergic activity in the dorsal striatum by means of pre-training injections of the antagonist haloperidol dose-dependently impaired classic radial arm maze learning by impairing some specific aspects of the maze behaviour These aspects include starting to visit an arm and to collect a food pellet, visiting a next arm and the development of an effective response pattern while collecting the pellets, neither general motor activity nor the number of revisits was altered

In sum, radial arm maze learning is partly sensitive to dorsal striatal dopaminergic inhibition

A brief general discussion is given in chapter 8, addressing three issues First, attention is paid to when dopaminergic treatment is most effective during the course of the learning and memory process The choice of the applied dopaminergic agents and its implications are explained The third and main issue concerns the question whether the observed effects after dopaminergic treatment support our hypothesis

Dopaminergic activity in the nucleus accumbens appears to play a specific role in the two tasks, in which external cues or stimuli are very important, namely social recognition of the juvenile based on olfactory stimuli from the anogenital region and spatial learning in the MWM based on relations between environmental stimuli, the dopaminergic activity in the dorsal striatum is not specifically involved in spatial learning in the MWM On the other hand, dorsal striatal dopamine has been shown to affect aspects of radial arm maze behaviour, that are not directed by external stimuli but structured by internal (intrinsic) factors In conclusion, the data from our experiments support the hypothesis of a differential role for dopamine in the ventral striatum (especially the nucleus accumbens) and the dorsal striatum in externally directed and internally structured aspects of learning and memory processes, respectively

SAMENVATTING

Dit proefschrift onderzocht de hypothese over een mogelijke differentiele rol voor dopamine in het ventrale versus het dorsale striatum in leer- en geheugenprocessen

Deze hypothese veronderstelt dat *ventraal* striataal dopamine betrokken is bij aspecten van leren en geheugen die gestuurd worden door *extern* (t o v het organisme) aanwezige cues, terwijl *dorsaal* striataal dopamine betrokken is bij aspecten van leren en geheugen die niet door externe cues, maar door *interne* factoren worden gestructureerd

De hypothese werd onderzocht in drie verschillende leer- en geheugentaken in de rat De uitgevoerde experimenten, die beschreven staan in de hoofdstukken 2 t/m 7, boden de mogelijkheid om de effecten van specifieke dopaminerge manipulatie op bovengenoemde aspecten te bestuderen

SOCIAAL GEHEUGEN (HOOFDSTUK 2)

De dopamine DA_1 agonist DPI werd geinjecteerd in de nucleus accumbens direct na de eerste ontmoeting tussen een volwassen mannelijke rat en een jonge soortgenoot Dit verlaagde (i v m een controle injectie) significant de duur (en frequentie) van het sociaal besnuffelen van de jonge soortgenoot door de volwassen rat tijdens een tweede ontmoeting, nadat een relatief lang interval was verstreken tussen de eerste en tweede ontmoeting Dit gegeven wordt geinterpreteerd als een verbetering van de herkenning van het jong ratje door de volwassen rat

De dopamine DA_1 antagonist ergometrine, geinjecteerd in de nucleus accumbens in combinatie met DPI op het juiste tijdstip, was in staat het effect van DPI te verlagen Een injectie van ergometrine alleen was ineffectief De verbetering van het geheugen door DPI blijkt dus dopamine specifiek te zijn

Onze resultaten vormen een sterke aanwijzing voor een geheugen verbeterende rol van de dopaminerge activiteit op het niveau van de DA_1 receptoren in de nucleus accumbens

SPATIEEL GEDRAG IN WATER-DOOLHOVEN (HOOFDSTUKKEN 3 T/M 5)

Systemische injecties van de dopaminerge antagonist haloperidol in lage doseringen hadden effect op de prestatie van ratten in het standaard Morris waterbad (aan het water ontsnappen via een verborgen platform) Deze effecten wezen op een betrokkenheid van dopaminerge activiteit in allocentrische spatiele localisatie (localisatie van een object in de ruimte op grond van relaties tussen andere objecten in die ruimte) In de gebruikte lage doseringen verslechterde haloperidol de locomotor activiteit in een open veld noch het vermogen om aan het water te ontsnappen via een goed zichtbaar platform (niet spatieel)

Vervolgstudies verschaften aanwijzingen voor een specifieke betrokkenheid van de nucleus accumbens Injecties van haloperidol in lage doseringen in de accumbens verslechterde op eenzelfde, dosis-afhankelijke manier het aanleren van de allocentrische spatiele localisatie van

het platform Ook nu gold weer dat de gekozen doseringen de locomotor activiteit in het open veld noch het leren te ontsnappen via een zichtbaar platform veranderden

In contrast hiermee gaven de resultaten van behandeling met haloperidol in het dorsale striatum een ander beeld te zien In een lage dosering had haloperidol geen effect op het allocentrisch spatiele leren, terwijl twee hogere doseringen het ontsnappen via zowel het onzichtbare als het zichtbare platform verslechterden (zonder een dosis-respons relatie in beide situaties) Dit gegeven suggereert dat de dopaminerge activiteit in het dorsale striatum geen specifieke rol speelt bij de spatiele localisatie in het Morris waterbad

Een hoge dosering van haloperidol, toegediend in de nucleus accumbens of in het dorsale striatum in goed getrainde dieren, had geen effect op de goede prestatie van deze dieren in de test trials

De betrokkenheid van het dorsale striatum in egocentrische spatiele localisatie (localisatie van een object op basis van relaties tussen het object en het organisme zelf (afstand en richting)) werd bekeken in een simpele, water T-doolhof In deze taak (niet beschreven in de Introductie, maar zie hoofdstuk 5) moet een dier leren te ontsnappen op een platform, dat te vinden is door altijd eenzelfde draai te maken in de T-maze (òf altijd links, òf altijd rechts), onafhankelijk van de omgeving In deze eenvoudige taak werd geen specifiek effect gevonden van een gekozen doseringen van haloperidol in het dorsale striatum op egocentrische spatiele localisatie

Samengevat toonden de resultaten een specifieke rol voor de nucleus accumbens in allocentrisch spatieel leren, terwijl het dorsale striatum alleen een niet-specifieke invloed uitoefende Terwijl de acquisitie wel beinvloed kon worden, werd het terughalen van goed geleerde informatie en een goed getrainde response niet veranderd door striatale dopaminerge manipulatie

RADIAAL DOOLHOF GEDRAG (HOOFDSTUKKEN 6 EN 7)

Veranderingen in de dopaminerge activiteit in het ventrale of dorsale striatum hadden geen effect op de prestatie van dieren die goed getraind waren in het oplossen van een simpele radiaal doolhof-taak, tijdens één test-trial

Daarentegen verslechterde inhibitie van de dopaminerge activiteit in het dorsale striatum, door locale toediening van de antagonist haloperidol vooraf aan de trainingsblokken, het leren van de oplossing van de klassieke vorm van de radiaal doolhof, door enkele aspecten van het gedrag in de doolhof te beinvloeden Tot deze aspecten behoorden het starten met bezoeken van de eerste arm en beginnen met het verzamelen van de voedselbrokjes, het bezoeken van elke volgende arm en het ontwikkelen van een vast en effectief respons-patroon tijdens het verzamelen van het voer, de algehele motor activiteit was niet verminderd en het aantal bezoeken aan reeds bezochte armen was evenmin veranderd

Concluderend is het leer-gedrag in de radiaal doolhof ten dele gevoelig voor verminderde dopaminerge activiteit in het dorsale striatum

In hoofdstuk 8 staat een korte algemene discussie weergegeven, waarin een drietal issues worden besproken Allereerst wordt onderzocht wanneer tijdens het leer- en geheugenproces behandeling met een dopaminerge stof het meest effectief is De keuze van de gebruikte dopaminerge stoffen en de implicatie daarvan worden toegelicht Het derde en voornaamste punt betreft de vraag of de geobserveerde effecten als gevolg van behandeling met dopaminerge stoffen in de gebruikte leer- en geheugentaken de voorgestelde hypothese ondersteunen

In de twee leer- en geheugentaken, waarin externe cues of stimuli een belangrijke rol spelen, nl de sociale herkenning van een jong ratje op basis van geurprikkels uit het anogenitale gebied en het spatieel leren in het Morris waterbad op grond van relaties tussen stimuli in de omgeving, blijkt de dopaminerge activiteit in de nucleus accumbens een specifieke rol te spelen, terwijl dopaminerge activiteit in het dorsale striatum geen specifieke invloed heeft op het spatieel leren Wel is gebleken dat dorsaal striataal dopamine invloed heeft op aspecten van het leergedrag binnen de radiaal doolhof, die niet door externe stimuli maar door interne factoren gestuurd worden

Concluderend ondersteunen de resultaten uit onze experimenten derhalve de hypothese aangaande een differentiele rol voor dopamine in het ventrale striatum (in het bijzonder de nucleus accumbens) en het dorsale striatum in extern gestuurde respectievelijk intern gestructureerde aspecten van leer- en geheugenprocessen

NAWOORD

Eindelijk is dit proefschrift nu gereed Op weg naar een dergelijk resultaat heeft menig promovendus wel eens het gevoel er helemaal alleen aan bezig te zijn Zo ook ik

Toch zijn er uiteraard veel mensen die onderweg hun bijdrage leveren, steun geven en zo het mogelijk maken dat het proefschrift er komt Een aantal van die mensen wil ik hier met name noemen

Allereerst heeft mijn promotor Lex Cools mij de mogelijkheid gegeven met het onderzoek te beginnen Geïnteresseerd in hersenen en gedrag ben ik op de afdeling binnengekomen als een leek op het terrein van de functies van dopamine en het striatale hersengebied In de loop der jaren heb ik over allerlei aspecten daarvan veel geleerd van mijn promotor, die op zijn voor mij onnavolgbare wijze gepoogd heeft mij te stimuleren De afdeling was altijd vol met mensen, met wie ik plezierig heb samengewerkt mijn mede-onderzoekers Bart Ellenbroek, Ruud van den Bos, Will Spooren, Eric Prinssen en Nijnke Rots, de medewerkers Mia Smeekens, Annette Willemen, Harry van Aanholt, Dick Heeren, Walter Hoeboer en Luuk Lubbers, de studenten Annette, Petra, Mariette, Helmy en veel anderen van wie ik helaas de naam kwijt ben (mijn geheugen is niet zo goed) Naast het werken herinner ik mij ook het kijken naar het Wimbledon toernooi en de Tour de France, leuke uitstapjes (met name het uitstapje dat ik mocht mee-organiseren maar waar ik door een motorongeluk maar voor een deel zelf aan mee kon doen) en goede voetbalpartijen Ik kan zeker zeggen dat ik in Nijmegen, in mijn eerste baan, veel heb geleerd over werken, samenwerken en het begeleiden van mensen

Soms nopen omstandigheden je weleens een andere koers uit te zetten Op de gedragsafdeling van het IMB aan de universiteit van Utrecht heb ik in de tweede helft van mijn project, niet geheel vrijwillig maar wel bewust, een tweede werkkring gezocht en gevonden Ik wil Berry graag bedanken voor de gelegenheid om bij hem te werken en voor alle ondersteuning die hij verleende bij de overgang Het was hier niet minder vol met mensen Ik noem en bedank mijn mede-gedragsonderzoekers Thorwald Hol, Erik van Doremalen, Hans Maaswinkel en Inge van Rijzingen, onze analiste Marlou Josephy en onze computerman Jacob Rousseau, de studenten bij Berry, waaronder Nicolette, en de grote chemiegroep van het IMB (teveel om op te noemen) Ik heb met heel veel plezier de kamer mogen delen met Marlou, bedankt De zeiluitstapjes met de gedragsgroep en de neuro-zeildagen voor het hele IMB waren natuurlijk ongeevenaarde evenementen Een andere omgeving geeft je overigens een nieuw zicht op de begrippen werken & samenwerken en het begeleiden van mensen

Ik heb ook veel geleerd van de twee studenten die hun stage combineerden met mijn wens tot de uitvoering van een aantal experimenten Mariette van Westerlaken in Nijmegen en Nicolette van Duursen in Utrecht Ik heb niet de illusie dat ik de stage van Mariette goed heb

begeleid, ik denk wel dat de stage van Nicolette veel beter ging. Ik bedank Nico voor de gezelligheid en haar enthousiasme.
Van de mensen op de achtergrond wil ik met name Angela en Erica van de stal noemen en Gerard Peek van de fotografie.
Ik ben blij met mijn paranimfen Ruud en Marlou.
Mijn vrienden wil ik bedanken voor de broodnodige aandacht en afleiding. Leny Lekkerkerk en Ruud hebben mij diverse malen de gelegenheid gegeven stoom af te blazen; bedankt.
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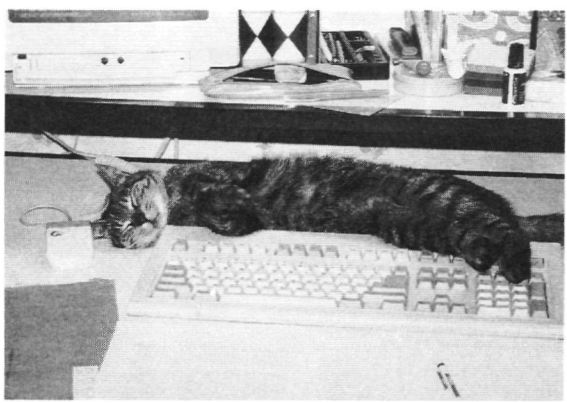

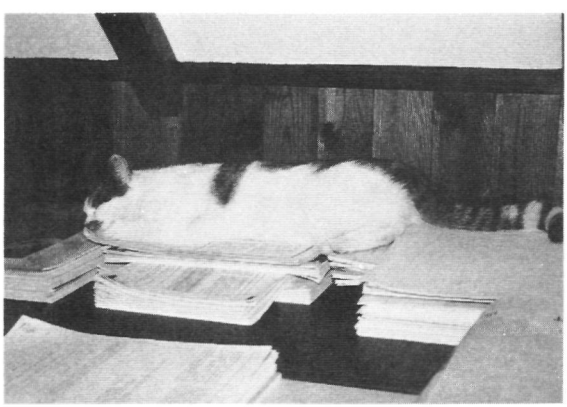

CURRICULUM VITAE

Geke Ploeger is geboren op 23 oktober 1961 te Ede. In 1980 is het diploma ongedeeld VWO behaald aan het "Oranje Nassau College" te Zoetermeer. In datzelfde jaar is ze begonnen met de studie biologie (oude stijl) aan de Rijksuniversiteit te Leiden. Anderhalf jaar later heeft ze deze studie, richting "Medische biologie", voortgezet aan de Rijksuniversiteit te Utrecht. De kandidaatsfase is in augustus 1983 met goed gevolg afgesloten. In november 1984 heeft ze het kandidaatsdiploma van de bovenbouw-studie filosofie behaald aan de Rijksuniversiteit te Utrecht. Vervolgens is de studie biologie weer opgenomen en is het doctoraal-examen hiervan, met het judicium 'cum laude', behaald in juni 1988, met als bijvakken Haematologie (Academisch Ziekenhuis Utrecht: prof. dr. J.J. Sixma en dr. Ph.G. de Groot) en Neurobiologie (Instituut voor Moleculaire Biologie: prof. dr. W.H. Gispen en dr. B.M. Spruijt) en als hoofdvak Ethologie (bij landbouwhuisdieren; Landbouw Universiteit Wageningen, vakgroep veehouderij: prof. dr. P.R. Wiepkema en dr. G.W.P. Schouten). Op 1 november 1988 is ze als Assistent-in-Opleiding begonnen met een 4-jarig onderzoek op de afdeling Psychoneurofarmacologie o.l.v. prof. dr. A.R. Cools aan de Katholieke Universiteit te Nijmegen. De laatste anderhalf jaar van haar project heeft ze doorgebracht op de gedragsafdeling van het Instituut voor Moleculaire Biologie (tegenwoordig opgegaan in het Rudolf Magnus Instituut van de medische faculteit) o.l.v. dr. B.M. Spruijt aan de Rijksuniversiteit van Utrecht. De resultaten van het uitgevoerde onderzoek staan beschreven in dit proefschrift.